American Association for the Advancement of Science

SCIENCE

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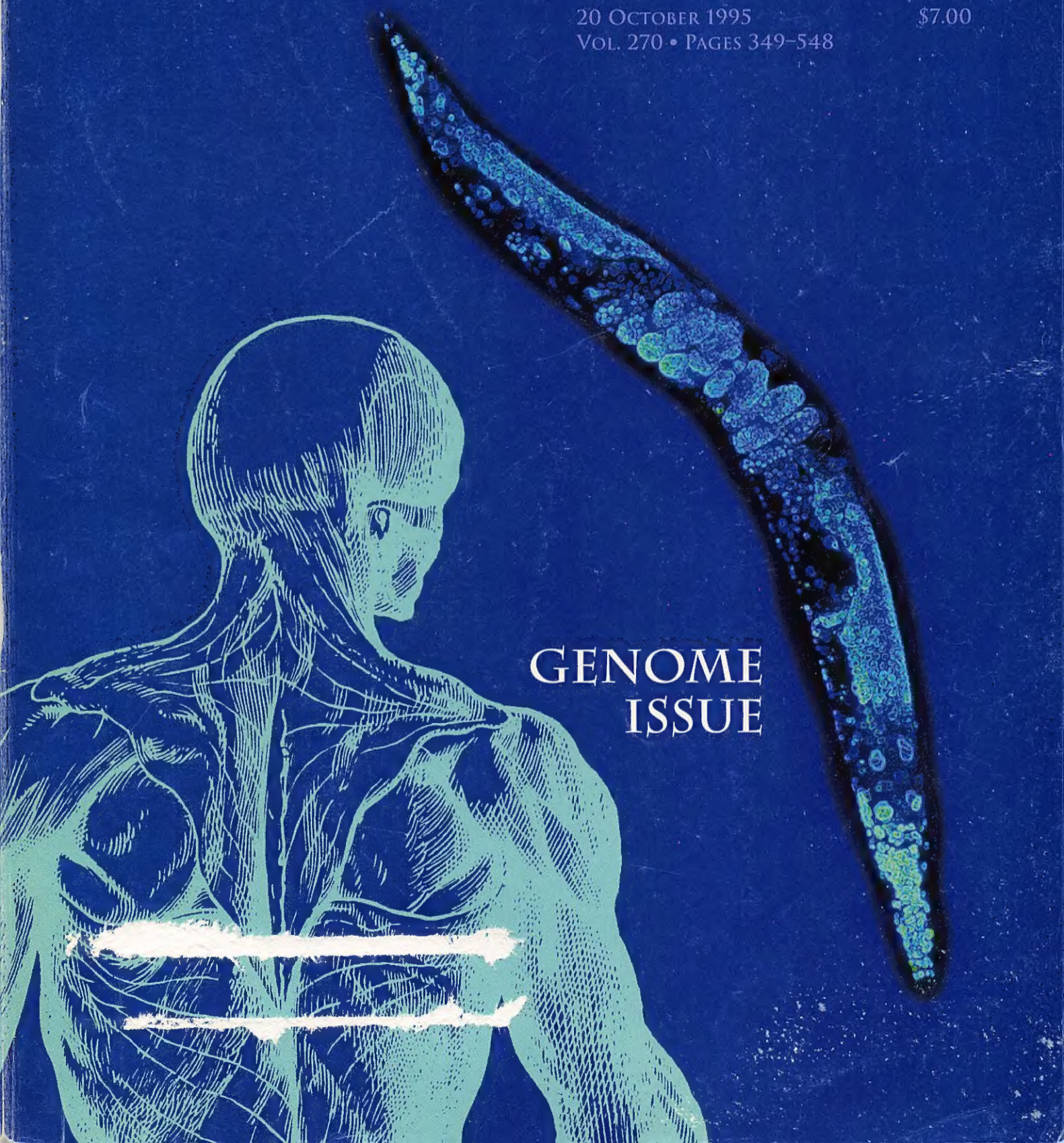

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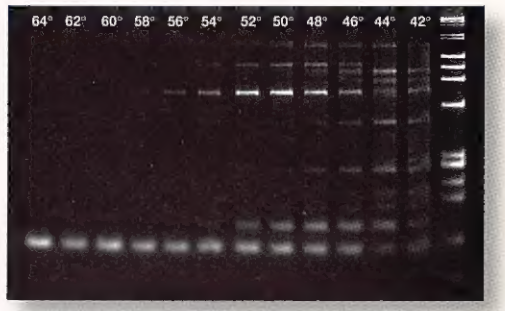

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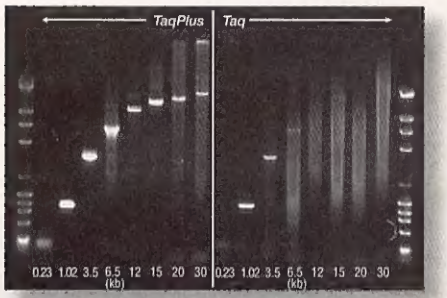

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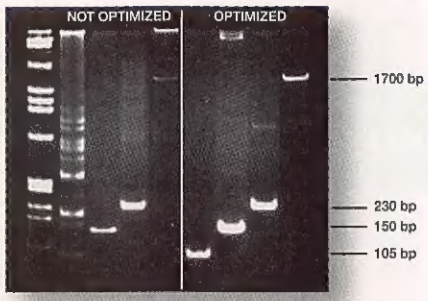

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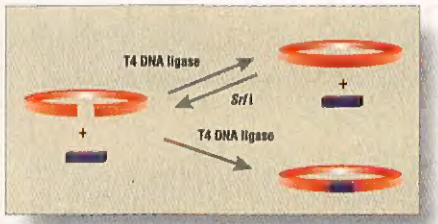

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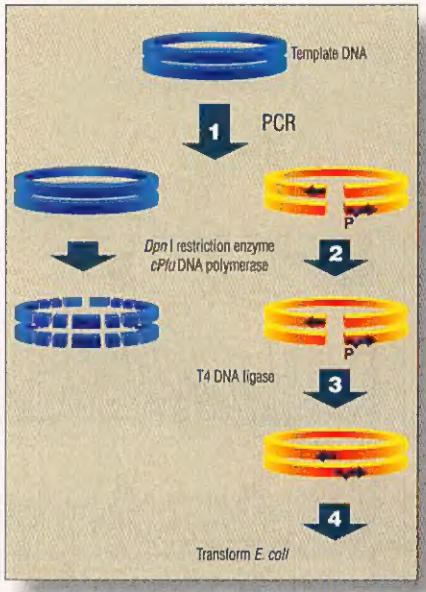

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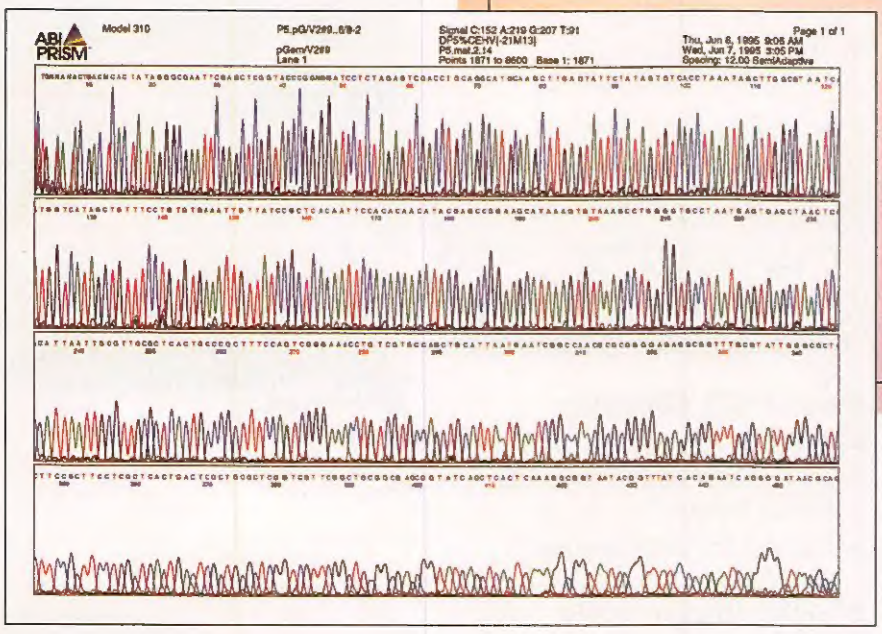
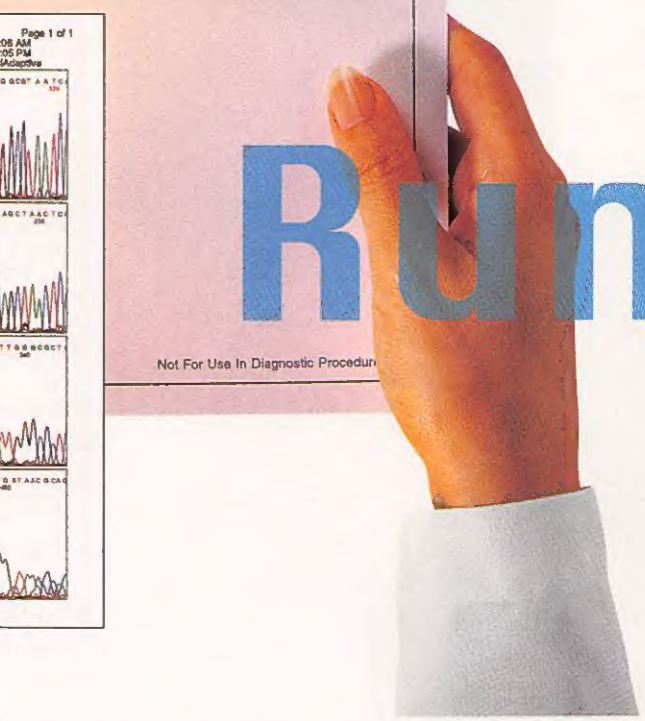


Dye/Sample Peak	Minutes	Size	Peak Height	Peak Area	Data Point
1F, 16	8.14	277.93	5418	19058	2198
1F, 24	8.67	432.72	4006	15278	2342
1F, 33	9.52	908.54	3903	22525	2571
1R, 17	7.82	204.00	6864	25369	2111
1R, 47	9.84	1740.00	3804	26539	2657
1R, 54	10.44	5117.00	2954	55230	2819



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AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE

380
The Nobel nine

447
Dates for fans and faults

NEWS

NOBEL PRIZES

GENOME ISSUE

POLICY FORUMS

ARTICLES

PERSPECTIVES

DEPARTMENTS

COVER

The Genome Project adds a new dimension to questions on gene expression in humans and model systems. A chart on page 415 summarizes progress in the *Caenorhabditis elegans* Genome Project and indicates some ways information about sequences can be used. News stories, Articles, Perspectives, Policy Forums, and Reports focus on technological developments, clinical applications, and ethical concerns resulting from the burgeoning of genomic information. [*C. elegans* image: F. Maduro and D. Pilgrim, University of Alberta]

REPORTS

TECHNICAL COMMENTS

397
Good things in small genomes

◩ Indicates accompanying feature

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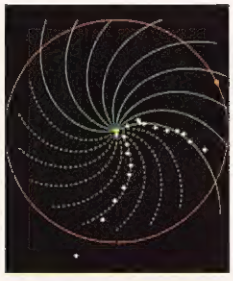
edited by PHIL SZUROMI

Fans and earthquakes

Prominent alluvial fans have formed along the faulted front of many mountain ranges in the western United States. By analyzing cosmogenic isotopes in boulders on fan surfaces, Bierman *et al.* (p. 447) dated fans along the eastern front of the Sierra Nevada in California, some of which were offset by the great 1872 Lone Pine earthquake. The data show that the surfaces of the fans were stabilized at least 8000 years ago, and that the recurrence interval of great earthquakes on the fault is 5800 to 8000 years.

Above the plane

Solar flares and other energetic processes inject electrons and ions into space, where they are guided by the solar magnetic field. Electron interactions with plasma can generate radio emissions (type II radio bursts) whose frequency decreases with distance from the sun. Such emissions are beamed along the solar system plane, so they are best observed from above or below the ecliptic. Reiner *et al.* (p. 461) report radio measurements obtained from the Ulysses spacecraft, which was put into an orbit that takes it over the poles of the sun. Such measurements allow coronal disturbances to be tracked as they move away from the sun, and they also verify theoretical predictions that the solar coronal magnetic field has an Archimedean spiral structure.



Hydrogen ecology deep underground

Nearly all known ecosystems on Earth, including those existing deep in the terrestrial subsurface, are somehow ultimately dependent on photosynthesis for energy. Stevens and McKinley (p. 450; see the news story by Kaiser, p. 377), however, describe bacterial communities in deep aquifers in the Columbia River Basalt Group in the northwestern United States. Their energy source appears to be hydrogen produced by reactions of water and rock in the basalt.

An old warm-up

Measurements of oxygen isotopes in ice cores have provided a detailed record of paleotemperatures at high latitudes during the Holocene; however, factors other than surface temperature may affect oxygen isotope ratios in ice. Because of heat diffusion from the surface, inversion of a borehole temperature record can provide an independent view of changes in surface temperature with time and can be used to calibrate the oxygen isotope thermometer. Cuffey *et al.* (p. 455; see the Perspective by McAyeal, p. 444) analyzed the borehole temperature record from the GISP2 ice core in Greenland. The temperature profile suggests that average temperatures increased by about 15°C from glacial conditions to the Holocene, an increase several times greater than that in the tropics.

Processed for export

Certain proteins, including several interleukins and types of fibroblast growth factor, are secreted directly from cells across the plasma membrane avoiding transport through the traditional secretory. This nonclassical secretory pathway for the production, processing, and transport of these important molecules has been very difficult to characterize in mammalian systems—in particular, the processing of precursors to mature, transport-competent proteins is not well understood. Adames *et al.* (p. 464) examined the export and processing of the yeast pheromone a factor, which is secreted by a similar nonclassical pathway. A yeast protease denoted Axl1p, related to the mammalian insulin degrading enzymes, played a critical role in the processing of the a factor precursor. The protease also played a role in bud site selection, but this function did not require an active protease. These findings not only bear on the classical pathway but also show that a single protein can regulate distinct signaling pathways—bud site selection and the mating response.

Gene expression by the numbers

Variations in the types and amount of genes expressed at different times in development, between normal and pathological states, or between different tissues can provide clues to gene expression. Two reports describe new methods for speeding the quantitative assessment of gene function (see news story by Nowak, p. 368). Schena *et al.* (p. 467) have developed a robotic system for rapidly generating high-density arrays of complementary DNA clones (in this case, 45 genes from *Arabidopsis thaliana*). By labeling samples with different dyes, differences in mRNA expression (such as between root and leaf) could be quantified. Velculescu *et al.* (p. 484) took a different approach to this problem which they call serial analysis of gene expression, or SAGE. They developed a system for producing concatenates of short diagnostic sequence tags; more than 1000 transcripts could be analyzed in a 3-hour period. Expression levels of tags corresponding to known genes were consistent with known mRNA expression, and new pancreatic transcripts were identified.

Gene therapy in SCID

A form of severe combined immune deficiency (SCID) results from defects in the gene for adenosine deaminase (ADA); deoxyadenosine then accumulates and is toxic to T cells. Bone marrow transplants can cure this disease but donors are usually not available; some symptomatic relief can be afforded by the use of enzyme replacements, such as ADA conjugated to polyethylene glycol (PEG-ADA). Two reports discuss the results of separate clinical trials in which the ADA gene has been transferred with retroviral vectors to two children with SCID during 2-year treatment periods. Blaese *et al.* (p. 475) targeted T cells and showed that vector integration and ADA expression persisted 2 years after gene treatment ended. Bordignon *et al.* (p. 470) used two different vectors to target peripheral blood lymphocytes and bone marrow cells. T cells derived from the marrow gradually dominated the population after treatment stopped. In each trial PEG-ADA was also given, but both reports conclude that gene therapy has contributed to therapeutic improvements in SCID.

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Figure 1.

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EDITORIAL

In Transition

In many ways, this has been a year of transition for the Genome Project, and this issue of *Science* highlights some of the changes that are occurring. Emphasis is shifting from physical mapping to large-scale sequencing. As more sequences become available, even databases of entire genomes, researchers are beginning to ask new kinds of questions and look for simpler and more rapid ways to convert nucleotide arrays into an understanding of gene expression. At an increasing rate, information about genes is being translated from the research lab to the clinic and is leading to growing concerns about the uses of genetic information. The Genome issue itself is undergoing some changes with the advent of electronic communication. The special chart in the issue will also appear in the "Beyond the Printed Page" section of *Science*'s home page (http://www.aaas.org/science/science.html). We also will offer electronic discussions in which readers can comment on the two controversial Policy Forums appearing this week. Finally, we will provide links to databases detailing the newly reported sequence of *Mycobacterium genitalium* and the physical map of chromosome 4 of *Arabidopsis*.

The effort to generate a physical map of the human genome has created its own momentum, but at what point should the community decide that the maps are good enough and that it is time to move on to the next goals? Maynard Olson believes that the time is right for a major drive to complete the sequence, but not all of Olson's colleagues will agree with the conclusion in his Policy Forum that the maps and technology are good enough to justify such a frontal assault, or that it is appropriate to divert resources from other projects.

Now that the second complete sequence of a free-living organism, *M. genitalium*, has been published (Fraser *et al.*), the field of comparative genomics is ready for a giant leap. The sequences of other small organisms will soon be available, as discussed by André Goffeau in his Perspective. Comparisons should provide insights into the basic systems necessary for life and for differentiated function.

It has been my experience that investigators who deal with model systems tend to be chauvinistic about their beasts. The authors of this year's wall chart were no exception. As one of the authors told me in the early stages of the project, "When this comes out, everyone will be suffering from worm envy!" For the genome community, the *Caenorhabditis elegans* project has been a paradigm of a group effort to reach a well-delineated goal. It is also a model system with great potential for exploration of developmental biology, cell biology, and neurobiology. The surprising finding that a *C. elegans* gene is a significant homolog of a human gene involved in early-onset Alzheimer's disease is an example of the potential insights into human disease to be gained from the study of model systems.

Demonstration of successful gene therapy in humans is one of the cherished ideals of the Genome Project. Although gene therapy has not yet cured any human disease, considerable progress has been made, as reviewed by Ronald Crystal. Long-awaited results (Bordignon *et al.* and Blaese *et al.*) relating to adenosine deaminase gene therapy for severe combined immunodeficiency are also described in this issue. These studies show that long-term in vivo expression of the transgene can safely be attained in patients, but the interpretation of clinical effects is not straightforward. It is important to emphasize that a clinical trial represents one step in the process, not the end of the road. Information may pass from clinical trials to research laboratories and back again many times before success is achieved.

The health care system in the United States is also in a time of transition and (hopefully) evolution as society discovers technological approaches to treatment of diseases but shudders at the associated costs. Increased costs mean that the prospect of loss of health insurance can be as frightening and damaging to a family as an illness itself. Members of a National Institutes of Health–Department of Energy Working Group and the National Action Plan on Breast Cancer have issued a set of recommendations in a Policy Forum by Kathy Hudson *et al.* for state and federal agencies trying to deal with the new dimension that genetic information adds to the health care problem. If these recommendations were followed, genetic information, including family histories, could not be used to establish insurance premiums or eligibility. Generating the sequences may prove to be the easy part; assimilating the implications of our genetic heritage in a way that will benefit individuals and society is the real challenge.

Barbara R. Jasny

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LETTERS

Fundamental issues

Francesco della Valle, founder of the now defunct Italian pharmaceutical company Fidia, asks how such companies can best interact with the academic world in a letter reacting to allegations by the Swedish newspaper *Dagens Nyheter* that Fidia tried to influence the Nobel Prize selection process. The editor-in-chief of *Dagens Nyheter* clarifies his paper's position. Other letters discuss a proposed genetic basis for aggression and sex differences in mathematics test scores.


DAGENS NYHETER.
Nobelprofessor knöts upp

Industry, Academia, and the Nobel Prize

I would like to clarify some points raised by Nigel Williams' article about *Dagens Nyheter*'s reporting on the Nobel Prize for Physiology or Medicine for 1986 (News & Comment, 22 Sept., p. 1663). In the original newspaper articles, *Dagens Nyheter* told about the campaign by the Italian pharmaceutical company Fidia and the efforts to influence Nobel decision-makers. We also said that Rita Levi-Montalcini was not a controversial choice as a Nobel laureate, and we were careful to stress that it was not possible to say whether the campaign had had any effect. When we told about Tomas Hökfelt's contacts with the Fidia company, his comments were given ample space in an adjacent article.

In my later remarks in the newspaper that you refer to (one statement, not two), I reiterated these points. Like my colleague Mats Holmberg, I also expressed my regret and concern that representatives of the Nobel committee had refused to be interviewed as part of our series and had only agreed to address our readers in an intervention of their own. I was also surprised that they stated so categorically that there had not been any campaign at all and by their unwillingness to discuss questions of principle and integrity that can arise with regard to contacts between business companies and scientists, however natural and necessary these may be.

I now see some of these distinguished scholars speculating about circulation wars among Swedish dailies and their relevance to investigative reporting. *Dagens Nyheter* and our colleague *Svenska Dagbladet*, with just a little more than half our circulation, are both subscribed morning papers. Our relative positions in the competitive market have been quite stable the last few years. Subscribed newspapers want to compete with quality and know that goodwill and prestige are precious values in that respect.

I was pleased to see that *Science* labels *Dagens Nyheter* "Sweden's most prestigious newspaper." It is our intention to remain just that.

Anders Mellbourn
Editor-in-chief,
Dagens Nyheter,
S-105 15 Stockholm, Sweden

Recent speculations about possible "corruption" in the selection of candidates for the Nobel Prize raise fundamental questions about the mode of interaction between the research-based pharmaceutical industry and the academic world.

What has been insinuated by discredited officials and misinterpreted by journalists as a "campaign . . . to promote the nomination of the Italian neuroscientist Rita Levi-Montalcini for the 1986 Nobel Prize for physiology or Medicine" (*1*) was in fact one of the many expressions of the effort that the pharmaceutical company Fidia, at the time led by me, put into creating an intensive and productive interaction with leading scientific settings, especially those engaged in so-called "fundamental research." It is increasingly becoming clear to the pharmaceutical enterprise, and it has been my firm personal belief for more than a decade (*2*), that fundamental research into the complex biological sequences operating in health and disease is making unprecedented progress and that scientific discovery in this area is a highly productive source of innovation in terms of potential new applications. More and more drug companies are now setting up collaborations with academia by engaging in "intensive research sponsorship."

Throughout the 1980s, Fidia was a name well known to neuroscientists worldwide. The Fidia-Georgetown Institute for the

Neurosciences in Washington, D.C., was established in order to increase the effectiveness of Fidia's research and development efforts. The Fidia Research Foundation sponsored top-level scientific meetings with prestigious lecturers, published authoritative books and journals, instituted awards and scholarships, and created a postgraduate International School for the Neurosciences. These initiatives were expressions of our wish to be as near as possible to the heart of scientific progress and were not even remotely thought of as an improbable means to "corrupt" scientists or to enhance obscure research data.

All of the above initiatives are now defunct, because of the unfortunate reversal Fidia took after I left the company in 1991, but the strategic concept is still very much alive. For more than 4 years, a select group of researchers has been working with me in a new health-care concern, Lifegroup, in intensified interaction with academic centers of excellence, foremost among them the group led by Levi-Montalcini. Her keen intuition about the role of nerve growth factor in the homeostatic interplay between the neurons and immune systems (*3*) led us to discover surprising new leads in immunomodulation and inflammation control (*4*).

As such successful industry-academic collaborations may come under scrutiny, I should like to invite debate about this critical issue. There appear to be no general rules or guidelines, either for academic researchers or for industrialists, about how to perceive and how to live with these interactions. What are the limits beyond which research sponsorship becomes too lavish? What should the independent researcher accept or reject in terms of collaboration with industry? Through public discussion of these questions, we may be able to reach a consensus, guaranteeing the kind of mutually rewarding interaction that both industry and academy pursue.

Francesco della Valle
Founder and Chairman,
Lifegroup S.p.A.,
Viale Tre Venezie 44/46,
35043 Monselice (PD), Italy

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Aggression in Mice and Men?

The title of the report "Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA" by Olivier Cases *et al.* (23 June, p. 1763) implies a correlation between aggressive behavior and the absence of monoamine oxidase A (MAOA) in mice, and its first sentences go on to draw a parallel with an hypothesized association between MAOA deficiency and "aggressive behavior" in men in a Dutch lineage (*1*). Yet Cases *et al.* describe phenotypic deficiencies in their mice ranging from "head nodding" and "trembling" to "moving backward," "frantic running," hunching, sleep abnormalities, and other developmental problems. Adults show abnormal swimming, hunched posture, and almost parenthetically we learn that they carry a retinal degeneration gene and are blind. Among all these massive and diverse deficits, Cases *et al.* draw attention to one, that resident males attack intruders faster than do mice with MAOA. This then becomes the focus of their statement that "aggression" in mice and men may be directly correlated to a specific gene deficit. Drawing causal or even correlative connections between complex socially defined behaviors and particular cellular and molecu-

lar processes is notoriously problematic. In a field fraught with implications for those concerned with the roots of violence in human society, caution is particularly required (*2*). Neither the title of the report nor its conclusions are adequately sustained by the complex behavioral data presented, and yet these have already been seized upon by the media—at least in the United Kingdom—and are likely to prove grist to the mill of ideologues and some politicians as supportive of biologically determinist views.

Steven P. R. Rose
Department of Biology,
Open University,
Walton Hall, Milton Keynes,
MK7 6AA, United Kingdom

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2. S. P. R. Rose, *Nature* **373**, 380 (1995).

Response: In our report we demonstrated that aggressive behavior and alterations in the cerebral cortex of the transgenic mouse line Tg8 resulted from the genetic loss of MAOA. The primary index of aggression was the bite wounds of Tg8 males housed in groups. Male mice of the C3H strain, similarly grouped, did not show such wounds. We emphasized the correlation between aggressive behavior and the absence of MAOA in our title and in the conclusion of our report to answer those who have suggested that aggression in MAOA-deficient men is merely a result of the "frustration" of being "in the middle of families of unaffected people" (Peter Breggin, as quoted by Charles C. Mann, Special Report: Genes and Behavior, 17 June 1994, p. 1689).

When listing several of the behavior traits of mouse pups, Rose omits mentioning that Tg8 pups bit the experimenter while C3H pups did not. With regard to the beam-walking test, the fact that Tg8 *and* C3H mice carry a retinal degeneration gene was stated in our report; blindness may be a factor contributing to the poor performance of the Tg8 mice. Thus Rose's rejection of our conclusions would appear to be less founded on our experimental results than on his own opinion (*1*, p. 381) that to describe dramatic changes in neurotransmitters "as if they were the cause of particular behaviours is to mistake correlation or even consequence for cause." Does Rose really believe that the "distress" of Tg8 pups causes the excess of serotonin (a ninefold excess in the brain at birth)? We maintain that this excess of serotonin is a causative factor of the pups' altered behavior.

Rose has also criticized (*1*, p. 380) the 1993 report by Brunner *et al.* (*2*) about abnormal behavior in men who are deficient in MAOA. Such men display retarded motor development, outbursts of threatening words and gestures, awkward sexual behavior, difficulties in task planning, and disrupted sleep. Much more rarely, excessive acts such as arson or attempted rape have been committed. Tg8 mice display abnormal motor development and disrupted sleep, aggressive behavior, altered sexual behavior, and altered reactions in new situations. We agree that experimental results must be examined scrupulously, but do not agree that such comparisons are in essence misleading.

Isabelle Seif
Olivier Cases
Institut Curie,
Centre Universitaire, Bâtiment 110,
91405 Orsay, France
Jean Chen Shih
Department of Molecular Pharmacology and Toxicology,
University of Southern California,
Los Angeles, CA 90033, USA
Edward De Maeyer
Institut Curie,
91405 Orsay, France

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■

Women, Math, and Test Scores

The article "Sex differences in mental test scores, variability, and numbers of high-scoring individuals" by L. V. Hedges and A. Nowell (7 July, p. 41) does not adequately address the question of mental test validity in predicting youth's performance in science and math courses or related careers. Studies have shown that standardized test scores tend to underpredict college grades for women and may overpredict for men (News & Comment, 17 Feb. 1989, p. 885); performance in school involves more than the ability to do well on standardized tests. In addition, the fairness of these tests for all populations, especially for girls and minority students, must be seriously questioned.

We find particularly disturbing the statement by Hedges and Nowell (p. 45) that "differences in the representation of the sexes in the tails of ability distributions are likely to figure increasingly in policy discussions about salary equity." Using standardized test scores as an argument for justifying salary inequity would be silly. It is job performance, not test scores, that should determine salary and advancement. This performance depends on many factors, including ability, education, training, work environment, and such personal qualities as motivation, commitment, and creativity. Test results do not predict on-the-job performance for the teacher, physician, lawyer, or scientist.

Finally, it concerns us that Hedges and Nowell suggest intervention for boys to raise their low literacy scores, but do not recommend interventions known to help girls reach their full potential in math and the sciences. Does this omission imply that girls cannot be helped? As women in science, we find this a dangerous assumption. Let us not exclude one-half of the population in our quest for math and science literacy.

*Penelope Kegel-Flom**
Catherine J. Didion†
Association for Women in Science, 1522 K Street, NW, Suite 820, Washington, DC 20005, USA

*President. †Executive director.

Response: We agree that test scores and group differences must be interpreted with caution, but Kegel-Flom and Didion may have misinterpreted our article. While we specifically suggested interventions for those with low literacy scores, we would also enthusiastically support interventions to help women improve their chances in mathematics and the sciences. We thought this was implicit in our positing socialization and opportunity structures as the likely cause of the differences we observed.

Our statement that test scores are likely to figure in policy discussions about salary equity was not a suggestion that test scores should be used to justify salary differences; it was a statement of fact that the amount of research on the relation between test scores and salary appears to be increasing. We agree that performance *should* determine salary and advancement. Social scientists, however, study real, as opposed to ideal or preferred, social behavior; their work may help document the discrepancy.

Finally, the issues of test fairness and job performance hold similar elusive qualities. A recent review (*1*) supports the notion that, because Scholastic Aptitude Test scores are somewhat *more* valid for females than for males, the linear regression prediction (derived from males' or from both sexes' scores pooled) tends to underpredict females' college grades. However, the higher validity coefficients for females also mean that females' grades are actually better predicted by tests than are males' grades.

Although there is extensive and convincing evidence that test scores predict job performance in many occupations (*2*), we know of no direct studies that have measures of on-the-job performance in the specific fields mentioned. Indirect studies of teachers (which use student learning adjusted for student background and school characteristics as a performance measure) show a consistent relation between verbal scores of teachers and performance. However, test scores can predict only a portion of performance in *any* occupation.

Larry V. Hedges
Amy Nowell
Department of Education,
University of Chicago,
5835 South Kimbark Avenue,
Chicago, IL 60637, USA

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Corrections and Clarifications

The News article "Another blow weakens EMF-cancer link" by Gary Taubes (29 Sept., p. 1816), discussed two papers that appeared in the October issue of the journal *Radiation Research*, not the "*Journal of Radiation Research*," as the article stated. The correct references are as follows: A. Lacy-Hulbert *et al.*, "No effect of 60 Hz electromagnetic fields on MYC or β-actin expression in human leukemic cells" [*Radiation Research* **144**, 9 (1995)] and J. D. Saffer and S. J. Thurston, "Short exposures to 60 Hz magnetic fields do not alter MYC expression in HL60 or Daudi cells" [*Radiation Research* **144**, 18 (1995)].

In the article "Grad school rankings rankle" by Wade Roush (News & Comment, 22 Sept., p. 1660), in the National Academy of Sciences (NAS) rankings of graduate geoscience programs (p. 1661), Stanford University appears twice—just as it does in the actual NAS data. The first Stanford listing is for its Program in Geophysics. The second is for the school's traditional geosciences program.

Figure 1 in the response by M. W. Moore *et al.* (15 Sept., p. 1591) to the technical comment "Neutrophilia in mice that lack the murine IL-8 receptor homolog" by D. E. Schuster *et al.* (15 Sept., p. 1590) was incorrectly placed in the text of the comment. It should have appeared in the text of the response by Moore *et al.*

The name of the fourth author of the report "Identification of a stimulator of steroid hormone synthesis isolated from testis" by N. Boujrad *et al.* (16 June, p. 1609) should have been given as Choong-Hyun Lee. In note 26 of the same report (p. 1612), the name of the Kyung Hee University was misspelled.

Figures 1 (p. 1314) and 2 (p. 1315) of the Research Article "Mutagenesis and Laue structures of enzyme intermediates: Isocitrate dehydrogenase" by J. M. Bolduc *et al.* (2 June, p. 1312) were printed as cross-eyed stereograms, not the usual wall-eyed stereograms. In the same article, Robert M. Sweet's affiliation should have been given as the Biology Department at Brookhaven National Laboratory, and in note 27 (p. 1318), it should have been noted that Sweet was supported by a grant from the U.S. Department of Energy.

Letters to the Editor

Letters may be submitted by e-mail (at science_letters@aaas.org), fax (202-289-7562), or regular mail (*Science*, 1333 H Street, NW, Washington, DC 20005). Letters will not be routinely acknowledged. Full addresses, signatures, and daytime phone numbers should be included. Letters should be brief (300 words or less) and may be edited for reasons of clarity or space. Beginning in October 1995, our previous policy of consulting with all letter authors before publication will be discontinued.

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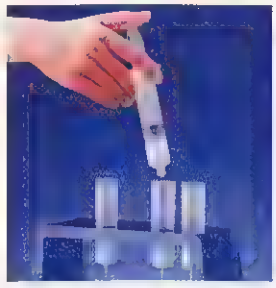

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edited by JOCELYN KAISER

Fund to Boost Latin American Science

Agricultural scientists in Latin America and the Caribbean face a twofold funding problem: Their cash-strapped governments have cut agricultural research 15% since the early 1980s, even as scientists' ranks have grown 22%, creating a money crisis. In response, the Inter-American Development Bank (IDB) is laying plans to rescue Latin American agricultural science budgets with a new regional fund for research.

The plan would pool $150 million donated by at least 15 beneficiary countries, along with $50 million from other countries and organizations, in an endowment that would distribute $10 million each year in peer-reviewed research awards to universities and national and international research centers. The work to be supported may include such areas as restoring nutrient-poor soil, using genetics to improve crop disease resistance, and managing fragile ecosystems.

IDB

Field work. New fund would support research such as efforts to enrich soil depleted by corn crops.

IDB Executive Vice President Nancy Birdsall, who presented the draft proposal earlier this month at a World Bank conference in Washington, D.C., says a key aspect of the fund is that "the beneficiaries are the contributors," guaranteeing good oversight of the technology projects. And Ruben Echeverria, senior economist of the IDB Environment Division, says the fund will have a "huge impact" by providing long-term funding and integrating research from multiple countries.

The IDB is seeking pledges of support from the 15 beneficiary countries in hopes of collecting $50 million by March. Echeverria says the bank could award the first grants early in 1997.

Congress Targets Superfund Research

Congress is poised to take a big slice out of the federal government's only program to carry out basic research on cleaning up hazardous waste.

At risk is the Superfund Basic Research Program (SBRP), set up in 1986 to support peer-reviewed university studies from contaminant transport to the toxicology of benzene. The money—$36 million this year—comes from industry and is passed through the Environmental Protection Agency (EPA) to the National Institute for Environmental Health Sciences, which this year funded 139 projects at 63 institutions. The EPA itself will spend another $60 million on Superfund research, but only on applied work. SBRP's director, William Suk, says his program is unique in its interdisciplinary approach and studies such as developing mechanistically based risk assessments.

But the program faces an uncertain future. A House-Senate conference in the next few days will try to agree on the program's 1996 budget, which the House wants to reduce by 12% and the Senate would slash by 56%, to $16 million. The Senate version "would be devastating," Suk says.

Also troubling is an authorization bill being drafted by a House Commerce subcommittee chaired by Representative Michael Oxley (R–OH). The draft bill doesn't mention funds for SBRP, which "[we] fear is an attempt to zero it out," says James Swenberg, who directs the SBRP program at the University of North Carolina. An Oxley staffer says the committee isn't trying to kill SBRP, but thinks industry should pay "just for cleanup," and that SBRP should be funded "through the normal appropriations" for EPA or the National Institutes of Health—unlikely given the current budget climate, Suk says. It's uncertain whether the House authorization would take effect until 1997, but "regardless of whose version you talk about," Suk says, scientists are worried.

U.S. Forensic Team Aids Bosnian Effort

Medical authorities in Bosnia are beginning the grisly task of identifying bodies in mass graves, says Moses Schanfield, director of the Analytical Genetic Testing Center in Denver and a member of a U.S. forensic team just back from the region. Schanfield estimates that less than one third of the missing civilians in villages he visited have been accounted for, based on the number of bodies recovered from two sites. In all, Schanfield says, 28 mass graves have been found, but he adds, "My guess is that there may be a hundred."

Schanfield took a week's vacation to join five U.S. scientists on a trip funded by AmeriCares, a charity based in New Canaan, Connecticut. The team included a pediatric osteologist, David Rowe of the University of Connecticut, and three forensic specialists who consulted for the prosecution on the O. J. Simpson case—Michael Baden, director of the New York State Police forensic sciences unit; Henry Lee, director of the Connecticut State Police forensics unit; and Barbara Wolf, director of anatomic pathology at the Albany Medical Center in New York.

U.S. researchers collaborated in setting up a DNA lab to help identify war victims at the Split Hospital in Croatia a year ago. The lab is running smoothly, Schanfield says, but the four-person pathology unit is overwhelmed by routine duties, war casualties, and the dead. Few exhumed bodies require DNA identification, but those that do are time-consuming. It is "emotionally draining" work, he adds, recalling how one young volunteer by chance dug up the remains of his own brother. The U.S. team has no plans at present to return to Croatia.

Advisers Champion Applied Research

With budget negotiations between the White House and Congress in deadlock, President Clinton's Committee of Advisers on Science and Technology (PCAST) has taken a stand—albeit a cautious one—on cuts proposed by the Republican Congress. The panel chaired by John Young urged Senate Majority Leader Bob Dole (R–KS) and House Speaker Newt Gingrich (R–GA) in a recent letter to continue funding "both basic and applied research and development."

It is applied research that is under fire from Republicans, who argue that efforts such as the Commerce Department's Advanced Technology Program amount to corporate welfare. PCAST, however, maintains that without federal support for such research through cooperative agreements between government and companies, "our industries will be at a significant competitive disadvantage, and our nation's strength will be diminished." The council reinforces that point in a set of principles accompanying Young's letter. The principles also strongly support fundamental research and science and engineering education, areas largely untouched by congressional budget-cutting. But the letter doesn't take issue with specific program cuts proposed for 1996.

PCAST members also have a message for Clinton, who was slated to give his first major science and technology speech 18 October at the White House. They urged him in a recent letter to take their principles into account in formulating his 1997 budget request, which the Office of Management and Budget is now putting together.

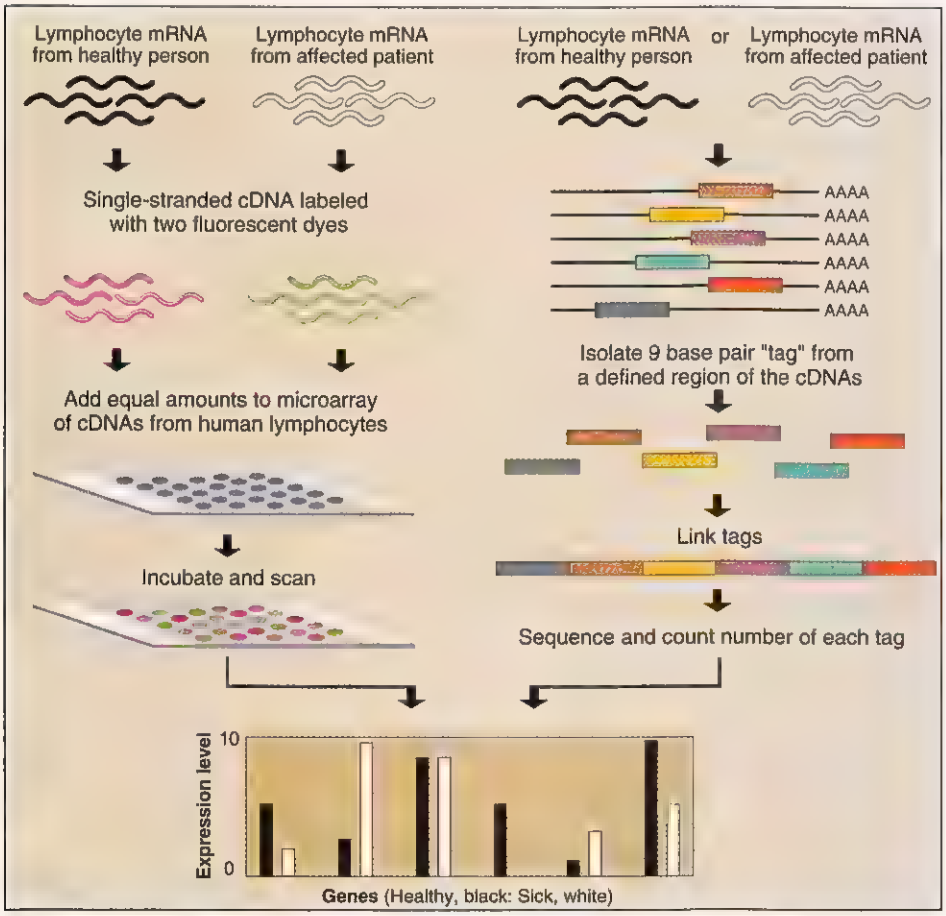
Entering the Postgenome Era

With the gene databases rapidly filling, the next step is to find out what the genes really do, either by measuring the activity of panels of genes—or by analyzing the cell's protein complement

Keeping an organism up and running is a feat of mind-boggling complexity. To transform a single fertilized egg cell into an adult human body and then keep that body alive and healthy, for instance, requires some 100,000 genes, each adjusting its activity to precise degrees and at precise times and locations. Thanks in part to the Human Genome Project, geneticists have done a remarkable job of assembling vast amounts of raw data about that intricate genetic machinery. Already unique sequences long enough to identify unequivocally well over half the genes are in the bag. Now, as some teams are gearing up for the final push to spell out all 3 billion base pairs of the human genome, others are poised to step into the postgenome era and find out how those genes act in concert to regulate the whole organism. Two techniques described in this week's issue of *Science* may help unveil the genes' multifarious roles (see pp. 467 and 484).

The best way to do that is to monitor the genes' fluctuating activities in different tissues at different stages of development, and in good health and in bad. But this is such a cumbersome job that, for the most part, until now it has only been possible to tackle one gene at a time. As a result, "we don't have a clue about the function of three fourths of the [identified genes]," says molecular biologist Mark Adams of The Institute for Genomic Research, a private research institution in Gaithersburg, Maryland, that has churned out huge numbers of partial gene sequences called expressed sequence tags (ESTs).

The new techniques, one developed by a research team led by molecular oncologists Kenneth Kinzler and Bert Vogelstein of Johns Hopkins University in Baltimore and the other by biochemist Patrick Brown of Stanford University in California, and his colleagues, aim to break up this logjam. They allow researchers to assess the activity patterns of thousands of genes simultaneously, generating in a matter of weeks information that might otherwise have taken years to gather.



Measuring gene activities. At left is a diagram of the microarray assay for gene expression; the SAGE technique is illustrated at right. Here, the procedures assess how gene expression differs in lymphocytes from a healthy person and those from a person fighting off an infection.

And geneticists are purportedly hungry for that knowledge: "I guarantee that in a year from now dozens of laboratories will have tried to adopt these approaches," says molecular biologist Jeffrey Trent of the National Center for Human Genome Research at the National Institutes of Health. "The value, the uniqueness [of the new approaches] is that they give a broad look at patterns of gene expression," he notes. This ability "is very, very important," adds molecular biologist Leroy Hood of the University of Washington, Seattle. "The future of biology is in the analysis of complex systems. And you can't look at [the expression of] one gene and understand how the system works." Molecular geneticist Mel Simon of Caltech in Pasadena agrees: "It represents a new era in the kinds of analysis we can do. It's a fantastic breakthrough for the study of the [genetic] mechanisms involved in development and in the control of differentiation."

Gene hunters are also likely to employ the new techniques in their frenzied quest to capture the crippled human genes that cause disease. Identifying genes whose activity is altered in diseased tissue will help researchers home in on those most likely to contain disease-causing mutations. And all these possibilities will not be lost on the pharmaceutical industry. Indeed, in anticipation of commercial interest, both teams have applied for patents on their techniques, which Hopkins has licensed to a biotech company called PharmaGenics Inc. of Allendale, New Jersey, and Stanford to a new company—Synteni Inc. of Palo Alto—founded last year by team member Dari Shalon.

The Hopkins team calls its technique Serial Analysis of Gene Expression, or SAGE. It relies on the fact that a sequence as short as nine base pairs is all it takes to identify 95% of human genes, provided the sequence is picked from the same place in all the genes surveyed. The amount of that sequence in a particular tissue is the measure of the gene's activity.

The Johns Hopkins workers demon-

strated SAGE's powers by using it to analyze the genes that are expressed in the human pancreas. They first extracted all the messenger RNA (mRNA), the products of active genes, from pancreatic tissue and copied it into complementary DNAs (cDNAs) that have the same sequences as the coding parts of the original pancreatic genes. During this step, the researchers also tagged the 3'- (far) ends of the cDNAs with a biotin molecule. After cutting the cDNAs into pieces with an enzyme called a restriction endonuclease, they were able to isolate the pieces containing each cDNAs' 3'-end with the aid of biotin-binding streptavidin beads.

Then, with a second restriction endonuclease, they clipped out a piece of DNA containing at least nine base pairs from those fragments. To complete the process, the researchers used the polymerase chain reaction (PCR) to create hundreds of copies of each short "SAGE tag," joined 30 to 50 different tags together in a single DNA molecule, and then cloned and sequenced these molecules. (The SAGE method includes a step that identifies any rogue tags that are preferentially amplified by PCR.) Because so many tags can be sequenced at one time, one technician, using a single state-of-the-art automated sequencer, can monitor the activity of 20,000 genes in as little as a month, says Kinzler. With the old methods, the same output would take years.

The technique shows not only which genes are active in a tissue, but also at what level. The Hopkins team reviewed the expression patterns of the pancreatic genes, by analyzing a total of 840 tags. (To improve sensitivity in actual experiments, thousands of tags would be screened.) Forty percent of these occurred as single copies, providing a baseline of the lowest level of detectable gene activity. But 77 tags occurred more than once, and, as predicted, the most abundant of those—one occurred 64 times and accounted for almost 8% of the RNA in the pancreas—encoded well-known pancreatic enzymes such as trypsinogen 2 and pancreatic lipase. "The number of times you see each tag is an index of the gene's expression," says Kinzler, who likens the SAGE tags to the ubiquitous supermarket bar code. The SAGE methodology, he says, "is the [genetic] equivalent of a cash register keeping track of the number of each item [a customer] buys."

"[SAGE] is very clever. You can get a large amount of information very rapidly," says Adams. "It offers the potential to small laboratories to do comparative studies and to take advantage of all the EST sequencing that's already been done." The pancreas experiment also demonstrated SAGE's power to help hunt down new genes. Several of the tags that occurred at high frequency in the

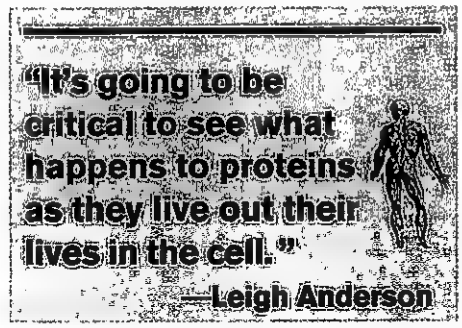
(continued on page 371)

MOLECULAR BIOLOGY

From Genome to Proteome: Looking at a Cell's Proteins

It sounds like Mission Impossible: Follow the changes taking place inside a cell—during embryonic development, for example—by identifying the thousands of different proteins the cell produces and watching how they ebb and flow over time. To a growing band of researchers, however, such a mission is becoming more realistic, thanks to the increasing volume of sequence data and to improved analytical techniques for proteins. Indeed, they believe such studies are a wave of the future for genome research and many areas of cellular biology, and have even coined a term for the emerging field: "proteome" research.

The growing interest in proteome projects comes as genome scientists are producing sequence data on more genes than they can put a function to. Some researchers are trying to find out what genes do by monitoring their expression patterns (see News story on p. 368 and Reports on pp. 467 and 484). But proteome researchers are approaching the task from the other end—looking at the proteins the genes produce. Although this approach is more complex and big obstacles remain, focusing directly on cellular proteins is "an important complement to studying DNA," says Jonathan Knowles, director of the Glaxo Institute for Molecular Biology in Geneva. "We will not [be able to] define disease mechanisms in molecular terms ... just using nucleic acids," he says.



That's partly because the level of gene expression is only one of the factors that determine how much of a protein is present in a cell. What's more, a gene sequence does not completely describe a protein's structure: After synthesis, proteins usually undergo "posttranslational modifications," such as addition of phosphate groups or removal of amino acids from the ends, and these changes can alter their activities.

But until recently, the advantages of focusing on proteins were overwhelmed by the difficulties. That is now changing fast, with the advent of powerful new methods of mass spectrometry that vastly simplify protein analysis, even on very small samples, and enable researchers to match them to their corresponding genes in the rapidly filling sequence databases. And, when protein studies "connect with what's known ... suddenly the whole approach has a lot more power," says yeast biologist Jim Garrels of Proteome Inc. in Beverly, Massachusetts.

Although the term "proteome" made its first appearance in the scientific literature only this year, in papers by Marc Wilkins and Keith Williams at Macquarie University in Sydney, Australia, the idea of analyzing the proteins expressed in different cell types has been around for nearly 2 decades. That was when Patrick O'Farrell, then at the University of Colorado, Boulder, developed two-dimensional gel electrophoresis, which made such an analysis possible. In this method, cell extracts are put onto a gel and the individual proteins separated first by charge and then by size. The result is a characteristic picture of 1000 to 3000 spots, each usually a single protein. In principle, these gel patterns reveal not only the amounts of proteins but also many of the posttranslational modifications they have undergone.

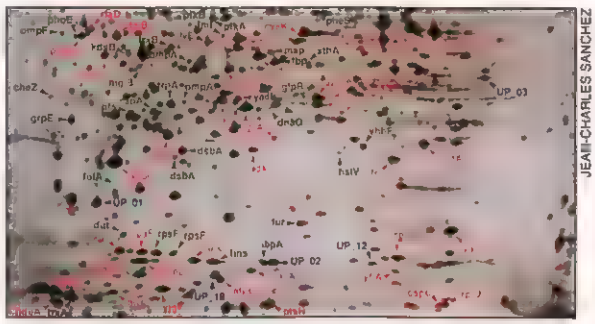
But by the mid-1980s, "people were getting discouraged," says Denis Hochstrasser, who heads the Clinical Chemistry Laboratory at the University of Geneva Hospital. Several problems had surfaced. Two-dimensional gels were not easily reproducible, making it nearly impossible to compare data from different labs. They revealed only the more abundant proteins in a cell—hardly a complete picture. Worst, says Hochstrasser, "it was very difficult to get information on the spots. It was like looking at the sky. You see the stars but don't know which ones they are."

Ironically, the worst problem is turning out to be the first one solved. The key to identifying a spot is to learn something about it that can be used to search protein databases. This first became possible in the 1980s after several groups developed methods to transfer spots from gels onto membranes so they could be analyzed. Amino acid composition, analysis of peptide fragments, and partial amino acid sequences can then often identify them as known proteins or products of predicted genes.

But it is the new mass spectrometry (MS)

methods—the "tool of the future," says protein expert Mike Waterfield of the Ludwig Institute for Cancer Research in London—that open the door to large-scale, automated spot identification. The two basic technologies—laser desorption MS, developed by Franz Hillenkamp at the University of Münster, Germany, and electrospray MS, devised by John Fenn at Yale University, both in the late 1980s—enable scientists to measure the molecular weights of large biomolecules easily and accurately, which in turn can help match proteins to those in the databases. Fenn's electrospray MS also generates partial sequences on purified peptides.

Still, it took several more years to bring the MS tools to the present stage, where they can begin tackling large-scale work. The crucial step—identifying proteins automatically and unambiguously—was just taken by Matthias Mann at the European Molecular Biology Laboratory in Heidelberg, Germany, and independently by Ruedi Aebersold and John Yates at the University of Washington, Seattle. Success was mostly a matter of getting more precise molecular weight measurements and developing more powerful database-scanning software, says Mann. His lab also devised methods for obtaining partial sequence data from unseparated peptides, making it possible to analyze mixtures of proteins at high sensitivity. Taken together, he says, all the improvements allow them to analyze some 10 proteins per day and identify the known ones with certainty.



JEAN-CHARLES SANCHEZ

MS marks the spots. Improved mass spectrometry helps identify the protein spots in 2D gels, like this one prepared from cells of the bacterium *E. coli*.

Besides being fast, MS methods have other advantages over conventional ways of analyzing proteins. They need far less protein—only one-tenth, or even one-hundredth as much, says Mann, and that allows identification of many of the less abundant spots on 2D gels. MS can also reveal many of the modifications to a protein's structure.

Researchers are now trying to scale up the MS methods so they can handle the thousands of proteins necessary for proteome work. For this step, the soon-to-be-completed yeast and *Escherichia coli* genome projects should provide the acid test. Once these genomes are fully sequenced, it should be possible, at least in theory, to link every 2D gel spot with the gene that encodes it, and with data on how the spot varies in different mutants and growth conditions. That, in turn, should help assign roles to many genes of unknown function—for example, by suggesting whether they belong to the same biochemical pathway or regulatory unit.

Indeed, a pilot project to test this approach is already under way, coordinated by yeast geneticist Piotr Slonimski at the Center of Molecular Genetics in Gif-sur-Yvette, France. The idea is to probe the function of newly discovered yeast genes and work out their interdependencies by disrupting them and then looking for changes on 2D gels.

So far, network members have analyzed 25 yeast strains, each with a disruption in a different gene. About half show changes in up to a few dozen protein spots, says Helian Boucherie at the Institute of Biochemistry and Cellular Genetics in Bordeaux, France, who is doing the 2D gel analysis together with Peter Mose Larsen at the University of Århus in Denmark. When these spots are identified—which should go quickly now that MS expert Mann is on board—they hope for a rich payoff in understanding the genes' functions. If the strategy works, the team will go on to a large study of 1000 genes.

Yeast biologists have high hopes for these efforts. "This is what people will want to do," says Garrels—"knock out their favorite gene and see its effect. It tells you a lot about the function of any gene you care to study."

But even though it's getting easier to identify protein spots on the 2D gels, not all the problems have been solved. First and foremost, says Jean-Charles Sanchez, who heads the 2D gel lab at the University of Geneva, is the difficulty in comparing gels from different labs—a frustrating problem now that there are over a dozen 2D gel databases on the World Wide Web. The difficulty is largely due to the gels themselves, Sanchez says, although he adds that things are improving as more labs adopt a method of fixing a pH gradient to the gel, eliminating some irreproducibility. Better software for comparing gel images also helps. Using the MELANIE II package developed by Geneva's Ron Appel, Sanchez could match spots on his *E. coli* gels to those in a database with valuable data on different mutant strains, which is maintained by Ruth VanBogelen of Parke-Davis Research and Frederick Neidhardt of the University of Michigan, both in Ann Arbor.

In parallel with these efforts, bioinformaticist Amos Bairoch and Appel have developed a system called ExPASy—the Expert Protein Analysis System—which links the Geneva 2D gel databases to the SWISS-PROT protein sequence data bank, and from there to many other databases and software tools. ExPASy, which receives some 300,000 queries monthly on the World Wide Web, also helps researchers guess a protein's function from its sequence. Bairoch is now working on predicting which sites in a protein are likely targets for posttranslational modifications—information that will help in interpreting 2D gels and understanding protein function. Last month, this work won Bairoch the SFr2 million ($1.7 million) Helmut Horten Foundation Award in biomedical research.

But for the Geneva team, the yeast and *E. coli* projects are only steppingstones to their real goal: using 2D gels as "clinical molecular scanners," says Hochstrasser. They pin their hopes on a growing body of data—collected in some half-dozen 2D gel databases worldwide—that link changes in gel patterns to specific diseases. Hochstrasser's unit is focusing on posttranslational modifications, which he calls "a major factor in disease." A case in point: Several cancer-causing oncogenes arise as a result of mutations in genes encoding enzymes that add phosphate groups to proteins. By studying such modifications closely, Hochstrasser predicts that researchers can develop diagnostic tools such as "marker" proteins for specific diseases or for assessing how advanced cancers are.

Scientists who like to think even bigger can look to the Large Scale Biology Corp. in Rockville, Maryland—which didn't get its name for nothing. Its mission, says President Leigh Anderson, is to harness the power of proteome studies for discovering new drugs and analyzing how they work. The idea is that drugs induce changes in protein patterns in the tissues of treated animals, and these changes give important clues about both the drug's desirable effects and its toxicity. For example, says Anderson, "if a drug inhibits cholesterol synthesis, we should see responses that identify it as active on cholesterol metabolism, and we do."

Anderson acknowledges there are still obstacles. Slow, laborious 2D gel methods remain a big problem, he notes, especially compared to the simple kits and procedures of DNA research. But he is optimistic that ongoing improvements and automation of 2D technology will make a big difference.

If these developments pan out, they will provide better ways to look at thousands of proteins on one gel, a feat even DNA researchers might envy. Then the key will be making sense of it all—a difficult task, but one Anderson says has to be faced. "It's going to be critical to see what happens to proteins as they live out their lives in the cell," he says. "There is no cheap, easy way around these problems. People are realizing that complexity has to be confronted."

–Patricia Kahn

(continued from page 369)

pancreatic tissue had no counterparts in the gene databases. Using the SAGE tags, the Kinzler-Vogelstein team identified the clones for those genes in a pancreatic gene library, sequenced the clones, and added the sequences to the database.

Brown and his colleagues reach the same endpoint as the Johns Hopkins team—a detailed description of gene activities in a given tissue or cell—but they get there not by sequencing gene fragments, but by using a miniaturized system that makes use of the fact that similar DNA strands bind or hybridize to complementary sequences. "Suppose you're [from] one of those many labs that have been madly sequencing cDNAs," says Brown. "You have sequences of tens of thousands of cDNAs, but little information about where they are expressed, and you want to find out very quickly." With their new "microarray" assay, he says, it's feasible to monitor the activity of thousands of genes per day.

For its proof-of-principle experiment, the Brown team turned to a weed called *Arabidopsis thaliana*, the fruit fly of plant genetics. Using a tiny computer-controlled two-pronged fork that they had designed specifically for the task, the researchers dropped onto a microscope slide spots of solutions, each containing a different double-stranded cDNA from an *Arabidopsis* gene library. After fixing this array of spots to the slide with heat and chemicals, the Brown team added pooled cDNA prepared from the protein-coding mRNA extracted from *Arabidopsis* leaves and labeled with a dye that glows red, and cDNA prepared from the protein-coding mRNA extracted from *Arabidopsis* roots and labeled with a dye that glows green. The spots where cDNA from the plant leaves or roots bound to the corresponding cDNA in the microarray fluoresce red and green.

The fluorescence patterns, measured by a computerized scanner, indicate the relative levels of expression of the genes in the two tissues, and the absolute activity of each gene can be determined by comparing its fluorescence to standards of known amounts of cDNA. Expression of some of the genes was 100-fold or greater in one tissue than the other, Brown says, "and when we sequenced them, it was exactly what you would have expected." For example, he says, the genes for photosynthetic enzymes were turned on in the leaves, but not the roots. In this initial test case, the microarray contained only 45 cDNAs, but since then the team has created microarrays with 1800 yeast DNA sequences, increasing the information gleaned from each experiment 40-fold.

Currently, both of the new techniques are in the prototype stage, and "it remains to be seen which technique will be more amenable to widespread use," says Trent. Nonetheless, he says, either technique—or one of the similar techniques coming down the pipeline—will be instrumental to the success of efforts to study how coordinated changes in the activity of batteries of genes convert undifferentiated cells into cells with specific tasks and attributes, trigger the responses of differentiated cells to radiation, hormones, or other outside stimuli, and drive healthy cells through the abnormal changes that end in disease. Other teams are making progress in developing techniques that allow them to assess directly what proteins are present in cells, although this work is not quite as far along (see p. 369).

Indeed, the two gene-expression techniques are already being put through their paces in real-life research situations. Both groups are trying to use them to spot the differences between normal and cancer cells. "As soon as we knew that SAGE worked," says Kinzler, the Hopkins team started a project to compare the activity patterns of genes in normal cells lining the colon with those in colon cancers. Kinzler expects definitive results within 6 months. Meanwhile, Trent and his colleagues, in collaboration with the Brown team, are using the microarray technique to search for the tumor-suppressor genes that may prevent abnormal, but not yet cancerous, skin cells from taking the final steps to malignancy.

Brown and team member Ronald Davis, also of Stanford University, have even bigger plans afoot. Sometime in 1996, when the sequence of the whole genome of the yeast *Saccharomyces cerevisiae* is complete, they intend to mass-produce microarrays containing the organism's entire suite of about 6500 genes. By studying changes in gene expression under different conditions—for example, when the nutrient-starved yeast produces spores, says Brown, "we will be able to see when the cells call different genes into action, and from that information generate new hypotheses about what the genes do."

Although the potential of having all this new information at their fingertips promises to make a geneticist's life more interesting, it is likely to generate another information glut, warns Kinzler. "Instead of the Krebs cycle," he says, referring to the complicated graphic depiction of the cell's major energy-generating system that adorns many laboratory walls, "we are now going to have expression maps of 100,000 different genes. Good luck figuring that out!"

–Rachel Nowak

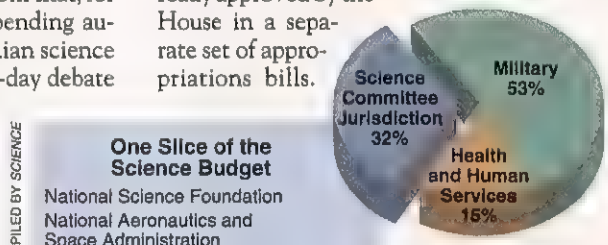
POLITICS

House Bundles 7 R&D Programs

The Senate is largely indifferent to it, the Administration is hostile, and it is unlikely to have any real effect on the 1996 budget. But last week the House passed a bill that, for the first time, lumps together spending authority for most nonmedical civilian science and technology programs. The 2-day debate leading up to the 248 to 161 vote provided a rare—and heated—discussion of federally sponsored research. In addition to putting science on center stage, it highlighted the widening gulf between the two parties on priorities for federal R&D.

Congressional action on civilian science and technology programs typically is scattered through the legislative calendar. This year, however, House Science Committee Chair Robert Walker (R–PA) championed a single bill that authorizes $21.5 billion—$3 billion less than current levels—for seven R&D agencies. "It's the first time we've focused attention on government R&D on the House floor," Walker told *Science*. "It makes more sense to look at science in a coordinated way." Congressional aides say the measure also demonstrates Walker's influence with the Republican leadership and advances his long-shot plan for a single Department of Science (*Science*, 31 March, p. 1900).

The funding figures in the omnibus authorization largely match the levels already approved by the House in a separate set of appropriations bills.



One Slice of the Science Budget

National Science Foundation
National Aeronautics and Space Administration
Department of Energy
Environmental Protection Agency
Commerce Department
- Technology programs (includes NIST)
- National Oceanic and Atmospheric Administration

U.S. Fire Administration

SOURCE: FIGURES COMPILED BY *SCIENCE*

One piece. The House reauthorization of R&D programs covers almost a third of the federal science budget.

Those bills determine 1996 budgets for agencies including the National Science Foundation, the National Aeronautics and Space Administration (NASA), the Environmental Protection Agency (EPA), the Department of Energy (DOE), and parts of the Commerce Department. High-ranking Democrats including Vice President Al Gore and Representative George Brown (CA) used the debate to lambaste Republican plans to cancel industrial research programs like the Commerce Department's Advanced Technology

Program and to restrict global change and anti-pollution research conducted by EPA and the National Oceanic and Atmospheric Administration (NOAA).

The bill, Gore said, "would hurt American workers, jobs, and living standards now and well into the future." The Office of Management and Budget warned that agency heads would recommend Clinton veto the bill "because of its unacceptably deep reductions" in a host of programs. Brown complained that Republicans were targeting nonmedical civilian programs and favoring defense and medical research, calling the bill "a first step toward the most significant postwar reduction in science funding ever proposed."

Walker disputes Brown's analysis, saying that the GOP bill preserves basic research and cuts what he calls corporate welfare—joint industry-government research programs aimed at developing technologies likely to be critical for high-tech industry. He also dismisses the threat of a Clinton veto. "The White House is saying it will veto everything," he says. "I can't take these veto threats seriously."

Democrats had little success in altering Walker's plan on the House floor. Brown's alternative, which would have boosted spending for the seven agencies to $25 billion, slightly above the president's 1996 request, was defeated, 229 to 177. "It's tough sledding," admits one Democratic staffer. At the same time, fiscally conservative Democrats, led by Representatives Tim Roemer (IN) and Bill Richardson (NM), failed to win support for cuts of up to 30% in DOE laboratory staffs. Freshmen Republicans fared no better with a proposal by Representative Scott Klug (R–WI) that would have forced Energy Secretary Hazel O'Leary to sell the department's civilian laboratories and consider privatizing Lawrence Livermore National Laboratory in California.

In the Senate, there is little support for an omnibus bill, and even individual authorizations are facing an uphill battle, with NASA's the most likely to succeed. But Senate passage of even one could lead to a conference between the House and Senate, giving Republicans a chance to send at least one science-related authorization to the president.

The real impact of the omnibus House bill may be the heightened visibility for federally funded research. And even though the Administration opposes the details, it sees merit in taking a broad look across federal science. "It's clearly a good thing," says one White House official, "because it allows you to make trades, to compare and contrast priorities." And at a time when issues like Medicare, welfare reform, and the budget deficit dominate political conversations, science advocates from both parties say they need all the publicity they can get.

–Andrew Lawler

NUCLEAR DISARMAMENT

Physicist Wins Nobel Peace Prize

A British physicist, campaigner for arms control, and the only person to quit the Manhattan Project on principle has been awarded the 1995 Nobel Peace Prize. Joseph Rotblat shares the honor with an anti-weapons group he founded 38 years ago, the Pugwash Conferences on Science and World Affairs. (For news of the scientific Nobelists, see p. 380.)

In awarding the $1 million prize last week, the Norwegian Nobel Committee praised Rotblat and Pugwash for working to "diminish the part played by nuclear arms in international politics" and trying to eliminate such weapons. It also wanted to deliver a pointed protest against recent testing of nuclear weapons by China and France, said Nobel committee chair Francis Sejersted, professor of economic and social history at the University of Oslo in Norway. Although one French politician declared himself "scandalized," the French government sent Rotblat its congratulations.

Rotblat began protesting the atom bomb even before the public knew it existed, according to a memoir he published of the event (*Bulletin of the Atomic Scientists*, August 1985, p. 16). In 1939, Rotblat, who had been studying the energy distribution of fission neutrons, was recruited to work on the Manhattan Project in Los Alamos, New Mexico. He says he participated only to deter the Germans, who had their own bomb project, from ever using such a weapon. In hindsight, he recognized that it was "folly" to imagine that this would have stopped Hitler.

One evening in 1944, according to Rotblat, the Manhattan Project's military commander, General Leslie Groves, casually mentioned that "the real purpose in making the bomb was to subdue the Soviets." This remark, and evidence that Germany had abandoned its own bomb effort, persuaded Rotblat in late 1944 that "the whole purpose of my being in Los Alamos [had] ceased to be." Rotblat asked for permission to quit, and immediately found himself accused of spying for the Soviets. The allegations, he wrote, were "rubbish," although he had broken security during the project by meeting and helping—without Army approval—a disabled friend in Santa Fe, New Mexico. According to Rotblat, the U.S. military used this protocol violation to pressure him into silence. His colleagues didn't learn for decades that he had left the Manhattan Project in protest.

CHARLES MILLER/AP/WIDE WORLD

Disarming winner. Joseph Rotblat quit the Manhattan Project and won a Nobel.

The experience "radically changed my scientific career," Rotblat wrote, for he realized that even the most esoteric research will find practical applications. In 1955, Rotblat drafted an appeal for peace addressed to all the world's scientists, signed by Albert Einstein, Bertrand Russell, and other intellectuals. It warned of the threat posed by thermonuclear weapons and urged scientists to find a way to prevent catastrophe.

This manifesto solidified into an institution after Rotblat organized a meeting of scientists and others in 1957 at the summer home of industrialist Cyrus Eaton, in Pugwash, Nova Scotia. "We tried to change the name," recalls William Epstein, a 30-year member of the Pugwash Conferences who later served as the United Nation's adviser on disarmament. "But people liked the sound of 'Pugwash,' and it stuck." In its Cold War heyday, Pugwash served as an unofficial channel for communication among weapons scientists and negotiators both in the Soviet Union and United States. In doing so, Pugwashers and Rotblat drew the ire of conservatives; the organization looked like a "vehicle for Soviet propaganda," as a Reagan Administration official said last week. Nonetheless, the organization supported technical talks that smoothed the way for a series of arms-control treaties, including most recently the 1992 Chemical Weapons Convention.

After quitting his job as a bomb designer, Rotblat conducted research in nuclear medicine. Colleagues cite his studies on autoradiography, the use of radioactive iodine as a diagnostic tool, and his debunking of a theory in the 1950s that nuclear fallout was responsible for rising infant mortality. But his greatest achievement, says physicist John Holdren, chair of Pugwash's executive council, has been "making it respectable" to believe that nuclear weapons can be abolished.

Now 86, Rotblat was "totally overwhelmed" by the announcement, says an aide, Thomas Milne of Pugwash's London headquarters, and Rotblat soon lost his voice from giving interviews. But he was able to communicate his intentions for the prize money: It will go into the Pugwash "peace chest" to further disarmament.

–Eliot Marshall

U.S. CONGRESS

House Weighs New Science Institute

House Republicans have been talking all year about the need to reduce the federal bureaucracy, and the Commerce Department has been their prime target. But they have been split over what to do with various orphaned programs (*Science*, 22 September, p. 1664). Last week, they finally came up with a single plan. It would preserve the bulk of Commerce's scientific programs by moving ocean and atmospheric research, weather operations, and technical standards work into a new organization called the National Institute for Science and Technology. Although the plan would create another government entity, "this is a consolidation," not a new bureaucracy, says Science Committee Chair Robert Walker (R–PA).

The proposal is a compromise between freshman lawmakers, who wanted to dismantle the department, and committee chairs like Walker, with jurisdiction over it, who wanted to tinker with but preserve most of its programs. The plan will be folded into a bill covering the entire government that Republicans hope to finish next month. But the debate is far from over: The Senate version may ignore the whole issue, while President Bill Clinton has vowed to veto any measure that eliminates the department.

Walker's careful language reflects the difficulty of finding common ground even among his House colleagues. "We're prepared to dig in our heels on this," warns Representative Dick Chrysler (R–MI), the freshman lawmaker who has championed Commerce's demise and won the support of the House leadership on the issue. At the same time, the freshmen have proved themselves unfamiliar with many of the science-related programs within the department. Chrysler, for example, testified at a recent Science Committee hearing that about 90% of all weather forecasting data comes from private sources. Under questioning, he admitted he did not know that most of the information actually comes from satellites operated by the National Oceanic and Atmospheric Administration (NOAA), which makes up half of Commerce's budget.

SAM KITTNER

War of words. Walker says plan would consolidate, not increase, bureaucracy.

Walker and other committee members eventually were able to convince their colleagues that setting technical standards, conducting research, and operating a weather forecasting system are important government roles, and that a new institute was needed to oversee those activities. "We'll end up with a program that is more science-oriented and that has a more defined mission," says Walker. The plan, however, would eliminate the NOAA corps and fleet, as well as the Advanced Technology Program within the National Institute of Standards and Technology. The compromise would turn Commerce's Patent and Trademark Office into a self-sustaining government corporation, reduce the 36,000 Commerce civil servants by one-third, and save $8 billion over 7 years, House lawmakers say.

Commerce Secretary Ronald Brown dismisses the plan as "political trophy-hunting at its worst," saying it would cost billions of dollars rather than save money. And NOAA Administrator James Baker says the current system "works well, so we don't see a need to create new agencies." That opposition, plus a Senate that is reluctant to act, could mean a short legislative life for the proposed institute.

–Andrew Lawler

ASTRONOMY

Laser Takes the Twinkle Out of a Star

Lawrence Livermore National Laboratory scientists Ken Waltjen and Claire Max stood quietly in the intense darkness of the Lick Observatory last week, their eyes straining to peer through the open ceiling into the midnight sky. A 120-inch telescope loomed overhead, with a strange new appendage stretching alongside it like a rifle scope. As 9 October melted into 10 October, a shaft of rich orange light pierced the night sky, and 127 actuators began clicking away at the base of the telescope, constantly adjusting the instrument's focus. The two colleagues beamed. "It's a day that I thought we'd never see," sighed Waltjen, an electrical engineer.

The reason for the excitement was the first integrated test of a new "adaptive optics" system designed to take the twinkle out of stars. The shaft of orange light was a laser beam shooting straight up from the observatory to a point 100 kilometers above Earth's surface, where it encountered a thin band rich in sodium atoms. By nudging some of those sodium atoms into an excited state, the laser beam created a point of light that acts like a guide star for a new breed of deformable mirrors at the base of the telescope. At first, the guide star appeared blurred by atmospheric turbulence. But a high-powered computer constantly compared the image to a model of what it should look like and sent 1000 signals a second to the actuators, mounted on the back of the 5 1/2 inch mirror, which bring the "star" into focus.

DIMO VOURIAS

Guiding light. Laser-generated "star" sharpens Lick's focus.

Once the guide star was crystal clear, astronomers turned their sights to their true stellar target nearby, knowing that the adaptive-optics system had already compensated for atmospheric distortions. In last week's test, University of California astronomers took data from young star formations that could not be seen without the new technology. Although Livermore scientists had created a guide star in 1992, the experiment was the first to use the system in a real observation.

The researchers involved were ebullient. The successful experiment, said project leader Max, means that old observatories like Lick, outside San Jose, California, could be made as good as the best in the world. And the best in the world, she said, could become as good as, if not better than, the $1.5 billion Hubble telescope, sent into orbit specifically to avoid the atmospheric turbulence that scientists now seek to nullify from the ground.

Several observatories are planning to install similar systems. Over the summer, Livermore scientists agreed to design an adaptive optics system for the 10-meter Keck telescope in Hawaii, the world's largest. A laser for Keck is also on the drawing board, and other laser guide star projects are under way at the University of Arizona and at Apache Point, New Mexico. Almost a dozen large telescopes are being planned worldwide, and each could some day sport a laser guide star to show the way.

–Jonathan Weisman

Jonathan Weisman is a science and defense writer at The Oakland Tribune.

ASTRONOMY

Did Galaxies Like Our Own Start Life in Chains?

Looking for the ancestors of present-day galaxies, says Lennox Cowie, "is sort of like looking at the Burgess Shale." Like the strange animal forms in that famous 550-million-year-old fossil bed, galaxies had "all kinds of baroque morphologies" in the cosmic past, from plasma-spouting radio galaxies and quasars to ill-formed dwarfs. Obvious ancestors to the stately spirals that fill today's universe are nowhere to be found. But by digging a little deeper into the strata—farther out in space and back in time—the University of Hawaii astronomer thinks he has spotted swarms of galaxies like our own in the process of formation.

The family resemblance isn't obvious, though: These protogalaxies look like strings of bright beads. Indeed, a few such structures had been spotted on earlier deep-space images, but they had attracted little notice. Only when Cowie and his Hawaii colleagues Esther Hu and Antoinette Songaila pushed their search for ancestral galaxies to fainter magnitudes with two of astronomy's most powerful instruments—the Hubble Space Telescope (HST) and the 10-meter Keck Telescope on Mauna Kea—did these "chain galaxies" proliferate and show themselves for what they are: massive galaxies like our own, furiously forming stars.

These claims, based on images made at the ragged edge of telescope resolution and detector sensitivity, have sparked controversy. Some galaxy watchers aren't sure that the chain galaxies are as common in the early universe as Cowie and his colleagues estimate in their papers, which appeared in yesterday's issue of *Nature* and in the October *Astronomical Journal*. Others doubt the ancestor-descendant link that the Hawaii group is claiming. "I'd like to reserve judgment," says Richard Ellis of Cambridge University. "It's premature to say that these are the ancestors of big galaxies like our own."

But still others think Cowie's group has filled a gap in cosmic history. As Craig Hogan of the University of Washington puts it, "We're on the threshold of accounting for all of the stars seen today. ... The conjecture is that if you took galaxies like these and waited 10 billion years, they would look like our own."

If Cowie and his colleagues are right, they will also have solved a puzzle posed by earlier studies of faint galaxies. For more than 10 years, flocks of faint blue objects had been showing up in the most sensitive images of the night sky. The blue color—the hue of hot, short-lived giant stars—showed that they were rapidly spawning new stars, and their faintness suggested that they lay at vast distances. Astronomers thought they might be seeing present-day galaxies in their infancy.

In the last 5 years or so, however, that assumption broke down. Detectors became sensitive enough for Cowie and others to collect spectra of these dim blue objects and measure the redshifts of spectral lines—an indication of distance. The redshifts averaged about 0.3, which put the galaxies no more than 3 billion or 4 billion light-years away, a result implying that their faintness is due to small size, not distance. Apparently, astronomers had been seeing only a tribe of blue dwarf galaxies that had come and gone in the recent past. "A year ago I was extremely confused about all this stuff," says Cowie.

Now, he says, "one is back to where one started." Many of the very faintest of these blue objects, he says, turn out to be massive and distant after all. The first evidence that ancestral galaxies might be hiding among the blue dwarfs came from the HST.

The HST's strength is its high resolution, which enables it to make images of objects that would be mere blurs from the ground. Cowie and his colleagues used exposures lasting as long as 8 hours to tease out the structures of faint blue galaxies. Most were shapeless and irregular, an appearance that other HST images have shown is typical of faint blue dwarfs. But at the very faintest magnitudes, says Cowie, "we started to pop out these ... truly bizarre-looking objects"—chain galaxies.

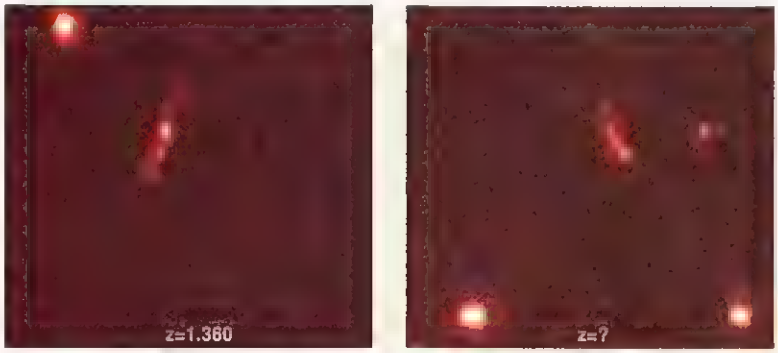
The string-of-beads appearance of the objects wasn't entirely new, but their numbers were; Cowie and his colleagues estimate that they account for as many as half the galaxies seen at blue-light magnitudes of 24, some 10 million times fainter than the faintest star visible on a dark night. The observers guessed they had found a whole new species in the galactic fossil beds. And when they aimed the Keck telescope at about 40 galaxies in this magnitude range—among them 12 of the chain galaxies seen by HST—their suspicions were borne out.

The Keck, with its prodigious light-gathering power and sensitive spectrograph, has an unmatched ability to dissect the light of a faint astronomical object to reveal its nature and distance. In the spectra of the faint galaxies, one feature stood out: an intense emission line from ionized oxygen. The position of the line on the spectra put the galaxies at redshifts of 1 to 1.6—perhaps 10 billion light-years away and two thirds of the way back to the big bang. And its strength compared to other lines in the spectra showed that they were making new stars at a frantic pace, says Cowie.

The line, he explains, is a signal of starbirth, because it comes from the gas surrounding young, massive stars, where intense ultraviolet radiation readily strips away electrons from oxygen atoms. Starbirth could also explain the beaded appearance of the galaxies, says Cowie. "When [a galaxy] starts making stars, it's not unusual to have it localized," creating bright knots. And starbirth at the rate implied by the line's intensity marks these galaxies as massive, or soon to be massive, he argues: "Even on the most conservative calculations many of these high-redshift objects are making stars at more than 100 solar masses per year." Keep that up for 100 million years—not long, by astronomical standards—and you will end up with a galaxy as massive as our own.

Cambridge astronomer Karl Glazebrook, who has studied faint blue galaxies, isn't fully convinced by the oxygen line. "It's not entirely obvious that these are the ancestors of massive galaxies," he says. Ellis agrees, adding, "My feeling is that these can be explained as a continuation of the star-forming [dwarf] galaxies that we see at lower redshifts." But Rogier Windhorst of Arizona State University (ASU) thinks it's hard to argue with the spectra: "Certainly these are massively star-forming galaxies."

What Windhorst and some others balk at,



Ablaze with star formation. The bright knots on these chain galaxies, seen with the Hubble Space Telescope, may be nests of new stars.

L. COWIE, E. HU, AND A. SONGAILA/UNIVERSITY OF HAWAII, INSTITUTE FOR ASTRONOMY

however, is the claim that chain galaxies are common in the distant universe, which would make them the standard ancestral form. Analyzing an HST image as deep as the ones Cowie studied, Windhorst says that he, ASU's Simon Driver, and their colleagues have seen a few chain galaxies—but plenty of other extremely faint objects, which are not chain galaxies, lying equally far away. Windhorst says he thinks Cowie may have found just one of many ancestral forms: "It's not the whole story."

Cowie agrees that his images show "a variety of wild and wonderful morphologies—including a fair number of chains but also some other weird beasts." But if his finding is at least part of the story, says Richard Griffiths of the Space Telescope Science Institute in Baltimore, galaxies like our own may finally be gaining a coherent history. "I think there's a sort of self-consistent picture taking shape that's more plausible than anything we've had for the last 20 years," says Griffiths. If so, astronomers may finally be glimpsing some order in the cosmic fossil beds.

–Tim Appenzeller

ASTRONOMY

Hints of a Planet Orbiting Sunlike Star

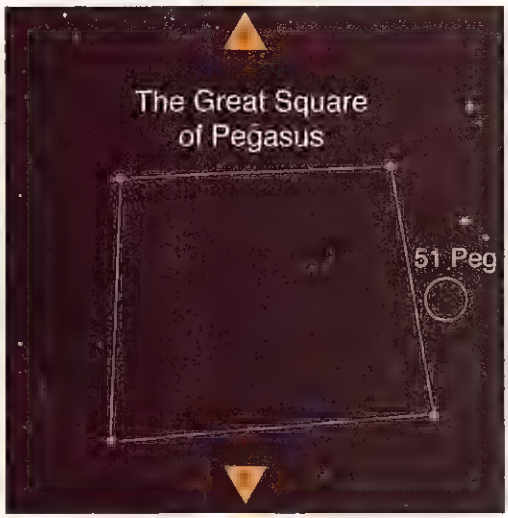
The Great Square of Pegasus catches many stargazers' eyes this time of year as the steed glides above the Northern Hemisphere's horizon. This year, however, it's going to attract not just star-watchers, but planet-hunters as well. Two astronomers at the Geneva Observatory in Switzerland, Michel Mayor and Didier Queloz, believe they have found the first planet outside our solar system that orbits a sunlike star. Their putative planet, at least half the mass of Jupiter, appears to be circling in a tight orbit around the star 51 Pegasi, visible just beyond the Great Square's leading edge. "The result is obviously incredibly exciting if it's true," says Philip Nicholson, an astronomer at Cornell University.

The excitement—tinged with some skepticism—isn't just because extrasolar planets have never been seen around a sunlike star, but also because standard theories suggest that planets this large don't form so close to stars. As a result, astronomers have been frantically casting about for information on the finding. The evidence is an apparent wobble in 51 Pegasi, which could be caused by a massive planet whirling around it with a period of about 4.2 days and an orbital radius of just one-sixth that of Mercury. Further details, however, have been hard to come by: Since they first presented their results at a conference in Florence, Italy, 2 weeks ago, Mayor and Queloz have declined further comment because a paper describing their findings is under review at *Nature*.

They did, however, authorize Douglas Duncan, an astronomer at the University of Chicago who is familiar with the work, to present an outline of their results at an astrophysics conference in College Park, Maryland, early last week. And *Science* has learned that astronomers Geoff Marcy and Paul Butler of San Francisco State University and the University of California, Berkeley, have already verified the detection of wobble—or "radial velocity"—of 51 Peg during a 4-day observing run on the Lick Observatory's 3-meter telescope just last week. The observations add up to "the first reliable detection of a planet orbiting another [sunlike] star," says Duncan.

The wobble the two groups have seen in 51 Peg is characteristic of the kind of motion an orbiting mass would produce. Like the balls of a bola thrown through space, all members of a planetary system whirl about each other, faster for the lighter planets and much slower for the heavier, central star. The star's wobble can be detected because atoms at the star's surface emit photons of light at discrete wavelengths, forming spectral "lines" that shift to shorter wavelength when the star is moving toward us and longer wavelength when it is moving away. Although the picture is blurred by the star's rotation, which causes photons emitted from different points to have slightly different wavelengths, Duncan says Mayor and Queloz managed see the wobble by monitoring about 5000 lines throughout the visible spectrum.

"The result is obviously incredibly exciting if it's true."
—Philip Nicholson



JIM SEE-EYE-FIS, ALDER PLANETARIUM & ASTRONOMY MUSEUM

Square dance. An apparent wobble in the star 51 Peg may be caused by a giant planet.

Mayor has been using these techniques to make "radial-velocity surveys" of dozens of stars for more than a decade, says Duncan. But 16 months ago the team upgraded its setup at the 1.9-meter telescope at Haute-Provence near Nice, France, isolating their spectrograph in a temperature-controlled room and operating it remotely via fiber optics. Then 51 Peg's wobble velocity of roughly 50 meters per second—much faster than would be produced by a lighter planet or one further from the star—leapt out of the data. But why wasn't the star seen by other groups with comparable or better accuracy making similar surveys? "That star was not being monitored by anyone else—including us," says Marcy, who says his measurements last week "confirmed everything [Mayor and Queloz] have discovered."

A canvas of half a dozen planetary scientists last week suggested that they will pore through Mayor and Queloz's data when they are published, looking for an effect that could be mimicking the signature of an orbiting planet. Among the possibilities: a previously unknown type of stellar pulsation; a large spot on the star's surface that might rhythmically suppress one side of the broadened emission lines, then the other, creating the appearance of a wobble; or an unseen binary companion whose orbit is seen nearly face-on from Earth. Although arguments can be made that each of these possibilities is unlikely, astronomers will "start out with a pretty skeptical view," says Cornell's Nicholson.

If the Geneva group's interpretation holds up, planetary scientists will have to confront a difficult question: How did the planet get there in the first place? Standard models of planetary evolution suggest that giant planets form with ice-and-rock cores much further away from a star. But Alexander Wolszczan of Pennsylvania State University, who last year found the first extrasolar planets orbiting a pulsar, is keeping an open mind. Wolszczan points out that his own discovery defied conventional thinking about where planets should exist. "I think it is important to be cautious about expecting, by default, to find an exact copy of the solar system," he says. "We should really keep our eyes open and expect anything."

–James Glanz

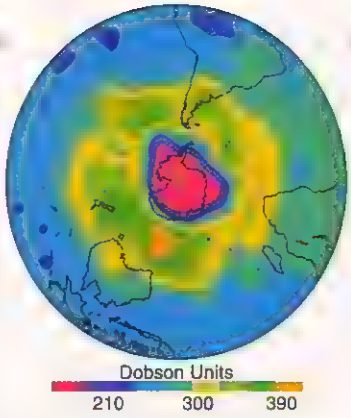
ENVIRONMENT

Ozone Hole Won't Worsen?

The timing was fitting. Last week, the chemistry Nobel Prize went to the researchers who first linked chlorine-containing pollutants with stratospheric ozone loss (see page 381). And last week brought the climax in the annual drama of Antarctic ozone destruction, which begins when the spring sun triggers the ozone-depleting reactions.

No one was quite sure what to expect from this year's ozone hole. Would 1995 finally show just a bit of healing, after several years when debris spewed by the 1991 eruption of Mount Pinatubo probably worsened ozone depletion? Or would the hole continue deepening year by year, as humanmade ozone-destroying chemicals continue building up in the stratosphere?

As of last week, the news was mixed. Satellite and balloon observations showed that the hole is about as deep and wide as ever. But new computer modeling of hole formation suggests a modestly upbeat conclusion: Future holes will be no larger. All else being equal, the model says, they should hold steady until early in the next century, when restrictions on the emission of ozone-destroying chemicals start to bring down their concentration in the stratosphere, allowing a 50-year-long recovery to begin.



NOAA

As bad as it gets? This year's ozone hole, as charted by TOVS.

Orbiting above the hole, the TIROS Operational Vertical Sounder (TOVS) gauged its area last week at about 21 million square kilometers, according to Arthur Neuendorffer of the National Oceanic and Atmospheric Administration (NOAA) in Silver Spring, Maryland. Although the hole may have had a little more growth left in it, Neuendorffer said, it probably would not exceed the 22 to 23 million square kilometers seen in recent years. Meanwhile, balloon-borne instruments flying into the hole from the South Pole found that total ozone had dropped from normal amounts of 280 Dobson units to as low as 98 Dobson units, say David Hofmann and his NOAA colleagues in Boulder. That equals the lowest ozone minima seen in recent years.

Expect about the same in the next few years, says atmospheric physicist Mark Schoeberl of the Goddard Space Flight Center in Greenbelt, Maryland. He and his colleagues have built a computer model of the swirling vortex of winds over Antarctica that forms the reaction chamber for the destruction of ozone. The model reproduces the year-to-year deepening and widening of the hole from 1980 to the early 1990s as ozone-depleting chemicals increased, Schoeberl says. As concentrations increase further in the next few years, however, the model predicts that Antarctic ozone "will go down faster each year, but [the hole] won't get deeper or wider."

The reason, Schoeberl explains, is that in the thin stratospheric layer where temperatures are frigid enough for the ozone-destroying reactions, depletion is practically complete already. The model predicts that factors such as the loss of ozone, which chills the stratosphere, will not enlarge the layer. The next few holes should tell whether the model's optimism is justified.

–Richard A. Kerr

Summaries of the status of the ozone hole are available at URL **http://www.wmo.ch/web/arep/ozobull.html**

PLANETARY SCIENCE

Hubbub at Saturn's Rings Revealed

Saturn's rings may look serene from a distance. But ever since the two Voyager spacecraft sailed by the planet in 1980 and 1981, planetary scientists have known that there's plenty of upheaval in the wispy F ring, which lies just beyond the main ring system. The F ring looks kinked, braided, and clumpy; what order there is seemed to be the work of two small "shepherd" moons, Prometheus and Pandora, that gravitationally herd the particles of the ring into line. Now a combination of new technology—the Hubble Space Telescope (HST)—and a rare opportunity to see past the glare of the other rings is revealing more players and deeper turmoil in the F-ring region.

Astronomers announced their first new F-ring discoveries at the end of July after Saturn's rings turned edge-on to Earth in May, momentarily reducing their blinding glare (*Science*, 4 August, p. 639). Using HST, Amanda Bosh and Andrew Rivkin of Lowell Observatory in Flagstaff, Arizona, detected a host of objects that did not fall where any known moons should have been. Now astronomers are starting to make sense of how the interlopers got there and what they are.

One of the summer's discoveries, temporarily designated S/1995 S4, is now clearly a previously unknown satellite orbiting just 6000 kilometers outside the F ring. But two others are actually Prometheus and a third nearby moon, Atlas, ranging far from their predicted positions. In Atlas's case, astronomers' uncertainty about its orbit might explain the straying, Bosh says, but Prometheus is another matter. "I'm inclined to think something has happened to Prometheus to shift its orbit," says Philip Nicholson of Cornell University.

NICHOLSON/NASA

Ring turmoil. One of HST's finds *(at left, right spot)* orbits near the F ring.

And Nicholson may have found that something. During August's second ring-plane crossing by Earth, he and his colleagues pointed HST at the F ring and discovered another new satellite, named S7, whose orbit appears to coincide with that of Prometheus. If the coincidence is real, 100-kilometer Prometheus may have a "sheep dog" companion about one-third its size that could explain its unexpected position. Like the so-called co-orbital Saturnian satellites Janus and Epimetheus, Prometheus and S7 would revolve around Saturn along paths perhaps 50 kilometers apart, the innermost and faster one slowly gaining on the slower until they approached each other. Then every few years, in a gravitational pas de deux, they would switch orbits, the pursued becoming the pursuer.

Alternatively, if the orbital coincidence is more apparent than real, says Nicholson, Prometheus's orbit may have shifted when it suffered a collision or near-miss with one of the largest unseen bodies thought to be embedded in the F ring itself. Dynamicists calculate that Prometheus's orbit did take it briefly inside the F ring in 1993. Such collisions might help explain the F ring's kinks and braids—as could the sustained gravitational tug from S7, from the shepherds, and from the moons embedded in the ring itself.

Astronomers will have one more chance to sort out the turmoil when the rings go edge-on to the sun on 21 November. Then it will be another 15 years before the next time Saturn's rings obligingly wink out of sight.

–Richard A. Kerr

The Saturn Ring Plane Crossing home page (URL **http://newproducts.jpl.nasa.gov/saturn/**) has the latest news and images.

MICROBIOLOGY

Can Deep Bacteria Live on Nothing But Rocks and Water?

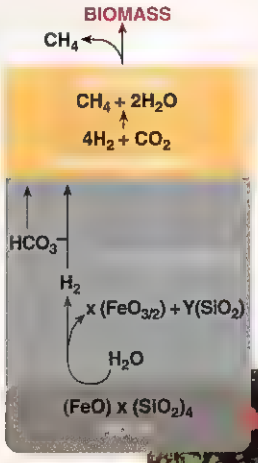
Nearly all living things owe a big debt to the sun. That's because the lowest link in the food chain consists of green plants and microbes that capture the sun's energy to produce food and oxygen. Even bacteria growing far beneath Earth's surface and at deep-sea hot springs have some tie to photosynthesis, depending either on organic matter or on oxygen. But on page 450, two researchers from the Pacific Northwest Laboratory in Richland, Washington, report that they've dipped more than a thousand meters into basalt rocks near the Columbia River and found bacteria that seem to get along fine without either. They live on rocks alone.

The microbes appear to get energy from hydrogen generated in a reaction between iron-rich minerals in basalt, a volcanic rock, and ground water. Hydrogen-eating bacteria aren't new, but all the ones found to date have depended on other microbes to make their hydrogen—or required oxygen to metabolize it. These organisms, in contrast, "don't need that first part of the food chain," says microbiologist Derek Lovley of the University of Massachusetts. "You just have the hydrogen consumers living off a geochemical reaction." If the laboratory evidence supporting the claim holds up, says Eugene Madsen, a microbiologist at Cornell University, "it [will] add another piece to the puzzle of what organisms can do and where they can do it." Some researchers think it might even shed light on the early evolution of life.

Even if the microbes turn out to get sustenance in a less unusual way, the finding still adds to a 15-year series of discoveries that have revealed bacteria in places where scientists once thought nothing could live. Microbes have been found living on organic matter in sediments hundreds of meters below the Pacific Ocean and in granite several kilometers down in Canada and Sweden. Others have turned up near boiling-hot vents in the oceans, where they metabolize sulfides from the Earth's crust with the help of oxygen, produced by photosynthesis, in seawater. Such findings led some scientists to predict the existence in deep rocks of microbial communities that subsist on geochemical energy alone.

And that's what microbiologist Todd Stevens and geochemist James McKinley seem to have found in ground water in Columbia River basalt aquifers at the Department of Energy's (DOE's) Hanford Site in eastern Washington. As researchers for DOE's Subsurface Science Program, they were surveying the area not for novel microbes, but to find out how bacteria might be affecting the composition and spread of the plumes of radioactive compounds and other pollutants in ground water beneath Hanford.

When the team drew water samples from wells up to 1500 meters deep in several aquifers, they found abundant bacteria—far more than they expected, because basalts contain little of the organic carbon that usually feeds bacterial growth. High levels of hydrogen and biologically generated methane suggested an active methanogenic community—anaerobic organisms that use hydrogen as an energy source to convert dissolved carbon dioxide to biomass, giving off methane as a byproduct.



Support for that hypothesis came when McKinley evaluated the ratio of carbon-13 to carbon-12 in carbon dioxide dissolved in the ground-water samples. Methanogens push up the ratio because as they metabolize carbon dioxide, they preferentially use up molecules containing carbon-12, which forms a more reactive bond. The ratios, McKinley found, pointed to the presence of methanogens.

The mystery was the source of the hydrogen they were living off. Most other anaerobic methanogens—common in such settings as freshwater swamps—get energy from hydrogen given off by other microbes. If that was true of these deep-living methanogens, they would still have a tenuous link to the rest of the biosphere. But the hydrogen levels seemed to be 1000 times higher than could be contributed by other kinds of microbes in the water.

A clue to its source came when a spark from a welder's torch set off an explosion of hydrogen gas in a nearby research well filled with basalt cobbles. "Those of us who had been studying the bacteria down in the basalt flows instantly made the connection," Stevens says. He and McKinley guessed that the basalt itself was giving off hydrogen when its ferrous silicates reacted with water.

To confirm their hunch, they mixed crushed basalt and deoxygenated water in the laboratory and found that the mixture did, indeed, generate hydrogen. Finally, they sealed ground water containing the bacteria together with basalt for up to a year and proved that they could get the bugs to grow in this unpromising medium.

Their results aren't entirely unexpected. "We knew it was only a matter of time before subsurface chemolithotropic [rock-eating] microbial communities analogous to the deep-sea hydrothermal vents would be discovered," Madsen says. But, he adds, "this … goes one step further by detaching such ecosystems from biogenic oxygen." Stevens and McKinley think this ecosystem could shed light on such fundamental questions as how organisms survived on Earth before the evolution of photosynthesis 2.8 billion years ago.

Some doubts remain, however. Lovley explains that many observers "are skeptical about whether this reaction of hydrogen production will actually take place" in nature. Stripping water of its hydrogen atoms requires reducing conditions—high in electron donors rather than acceptors—"way [beyond] anything anybody else has seen" in the Earth's crust, says Arthur White, a geochemist at the U.S. Geological Survey in Menlo Park, California. And although the reaction appears to take place with freshly crushed basalt in the laboratory, it might not occur on old rock surfaces underground. If it doesn't, the bacteria might be getting their hydrogen from a volcanic vent—still a diet of rocks. Or they might be relying on some hidden biological source, in which case they would not be so remarkable after all.

Stevens acknowledges that he and McKinley have "several lines of circumstantial evidence." To make a stronger case, he says, they need to find out the mechanism by which basalt donates the electrons to split water. They also want to know whether the microbes themselves promote hydrogen generation, perhaps by producing a chemical that erodes the basalt surface. These bacteria, after all, seem to be masters of self-reliance.

–Jocelyn Kaiser

T. STEVENS

Making do. Deep-living bacteria—red in the fluorescent-labeled image—survive on basalt *(green)*, perhaps by metabolizing hydrogen liberated from ground water. (Scale bar equals 5 μm.)

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edited by CONSTANCE HOLDEN

NIH's Radiation License Challenged

A visiting biologist from China, claiming that someone deliberately spiked her food or water with a radioactive tracer (phosphorus-32) while she was working at the National Institutes of Health this summer, is asking the federal government to suspend NIH's license to use radioactive research materials.

Maryann Wenli Ma and her husband, Bill Wenling Zheng, also a biologist, claim that NIH's poor control of lab reagents contributed to an episode this summer when someone apparently contaminated drinking water and possibly Ma's lunch in one of the NIH buildings with P-32 (*Science*, 28 July, p. 483). Last week the couple petitioned the Nuclear Regulatory Commission (NRC) to lift NIH's license until it agrees to put tighter controls on radioactive reagents.

According to the couple's attorney, Lynne Bernabei of Washington, D.C., they have made no decision about whether to sue NIH for damages. NIH Deputy Director Ruth Kirschstein has conceded in a statement that Ma and 26 other NIH staffers were affected by an "apparently deliberate act." But she said NIH has an "excellent" record of managing radioactive reagents and rejected Ma's claim that she and her child were at risk, saying "There is no reason to believe that Dr. Ma [who is now 8 months pregnant] has been injured or her pregnancy compromised by the amount of radioactivity to which she was exposed."

Ma and her attorney, however, are disputing NIH's estimate of her radiation exposure, which NIH says was below the annual occupational exposure limit of 5 rem. Ma has hired a consultant who claims that, correcting for technical errors by NIH, the real dose was about 9 rem. In connection with her allegations about sloppy safety practices, Ma also alleges that her supervisor, John Weinstein, was pressuring her to get an abortion to avoid delaying her research. Although Weinstein obtained a pregnancy declaration form for her, required by radiation safety rules, she claims he "coerced" her not to submit it. Weinstein flatly denies the allegations.

The charges will all be thrashed out in coming months as the FBI, the NIH, and the NRC all continue to investigate what an NIH official calls "one of the strangest occurrences we have ever had to deal with."

TOP TEN IN MATERIALS SCIENCE, 1990–1994

Rank	Institution	Citations	Rank	Institution	Impact
1.	IBM Corp.	1818	1.	Carnegie Mellon U.	34.1
2.	UC Santa Barbara	1174	2.	U. Illinois, Urbana	32.6
3.	AT&T Bell Labs	1095	3.	Lawrence Livermore NL	31.5
4.	Oak Ridge Nat'l Lab	866	4.	Lawrence Berkeley Lab	30.7
5.	Tohoku University*	753	5.	Kyoto University*	30.3
6.	Argonne Nat'l Lab	663	6.	UC Irvine	29.8
7.	Stanford University	663	7.	Argonne Nat'l Lab	28.8
8	Nat. Inst. Stand. & Tech.	658	8.	Stanford University	28.8
9.	U. Illinois, Urbana	651	9.	Harvard University	28.1
10.	Naval Research Lab	645	10.	Oak Ridge Nat'l Lab	27.1

*Japan. All other institutions are in the United States.

Citations matter. IBM is the world's heavyweight when it comes to production of high-impact papers in materials science, according to the latest tally by the Institute for Scientific Information (ISI). It garnered 1818 citations in the first 5 years of this decade and ranked 17th in citation impact (it got 26 cites per paper). ISI also reports that the most oft-cited author—with 624 citations of 19 papers—was A.G. Evans of the University of California, Santa Barbara, who studies characteristics of composite materials. The rankings were based on the 300 papers in each of the last 5 years that were cited most often in 150 journals. For further information, e-mail **cking@isinet.com**.

VICTOR ENGLEBERT/PHOTO RESEARCHERS

Making way for cropland. North Perimeter Road, Roraima, Brazil.

Burning Questions

Fieldwork for the first large-scale assessment of how Brazil's slash-and-burn agriculture is affecting Earth's atmosphere has recently been completed by a team of three dozen U.S. and Brazilian scientists.

While greenhouse gases produced by heavy industry have been studied to a fare-thee-well, says expedition member Peter V. Hobbs, an atmospheric scientist at the University of Washington, little is known about the consequences of burning forests, mostly done to clear cropland, a process that accounts for about one quarter of global CO_2 emissions. Brazil contributes about 20% of that quarter.

Scientists have attempted to construct models, but they have been fatally flawed because "we have not had reliable, consistent long-term data sets," says Robert Dixon, director of the U.S. Country Studies Program, which is funded by the U.S. Global Change Research Program. "There's been a lot of work on emission factors of factories and homes, but we have just a handful of meaningful data sets for forests."

The Brazil experiment, SCAR-B (for smoke, cloud, and radiation in Brazil), should change all that. It involved flying specially equipped planes over a 1500-square-kilometer swathe of Brazil that included everything from dry savannas to Amazon rain forests. The team studied the physics and chemistry of fire smoke from the ground up using a low-flying University of Washington plane, high-flying aircraft from the U.S. National Aeronautics and Space Administration, and satellites.

The measurements from these forays will for the first time provide detailed data on the amount of various greenhouse gases and particulate material emitted by burning timber, including variations for wet and dry wood, smoldering and flaming fires, and for different types of vegetation. SCAR-B also gathered data on a paradoxical notion—that burning vegetation hastens not only global warming but also global cooling, by fostering the buildup of dense smoke and clouds which deflect sunlight. Data from this expedition should be available for modelers starting within the next 2 years.

The Un-Nobels

The ballerinas were wearing lab coats, Harvard astronomer Robert Kirshner delivered his Heisenberg Certainty lecture in reverse, and for the fifth year in a row, the annual Ig Nobel Prize Ceremony beat the Nobel Assembly in Stockholm (see pp. 380–383) to the punch.

The editors of the Cambridge, Massachusetts–based *Annals of Improbable Research* (*AIR*) took over a Harvard University lecture hall on 6 October to award Ig Nobels in 10 categories, from literature to dentistry. This year's theme was deoxyribonucleic acid, highlighted by the biochemical ballet, "The Interpretive Dance of the Nucleotides." Nobelists including physicist Sheldon Glashow, chemists Dudley Herschbach and William Lipscomb, and biologist Richard Roberts played adenine, thymine, cytosine, and guanine at center stage as dancers swooped and spiraled around them, recreating the timeless rhythms of recombination. The scientist-performers appropriately worked up quite a sweat. As emcee Marc Abrahams, editor of *AIR*, said later, "Progress depends on geniuses, crackpots, and people who are just 99% perspiration. Surely all of them deserve to be honored."

Nine Make the Nobel Grade

This year, Nobel Prizes in science and economics went to pioneers in developmental biology, atmospheric chemistry, and rational behavior, and to the discoverers of two elementary particles

FLY DEVELOPMENT WORK BEARS PRIZE-WINNING FRUIT

Does the family of genes that determine where an insect sprouts wings also tell fish where to grow fins? Are the molecules that map out the body plan in an embryo later required to produce specific organs and tissues and to help cells communicate with each other? Today questions such as these preoccupy developmental biologists around the world, yet few researchers would be asking—and answering—them had it not been for pioneering studies done between the 1940s and the 1970s showing how a few genes control the embryonic development of fruit flies.

Now the Nobel Assembly in Stockholm has honored the scientific pioneers responsible for these studies. This year's Nobel Prize in physiology or medicine went to developmental geneticists Edward B. Lewis of the California Institute of Technology (Caltech), Christiane Nüsslein-Volhard of the Max Planck Institute for Developmental Biology in Tübingen, Germany, and Eric Wieschaus of Princeton University, all of whom identified genes that affect development in the fruit fly *Drosophila melanogaster*.

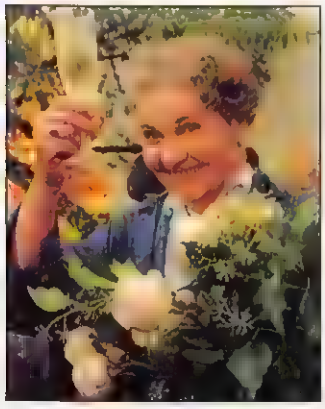
The selection is the first honoring basic developmental research since 1935. The field "hasn't been recognized very much" by the Nobel Assembly, says Nüsslein-Volhard, but now the award will help her spread the message that "basic research ... is really worth doing. And if you have a Nobel Prize I guess people listen a bit more carefully to what you say." More than just a capstone to a career in science, adds Lewis, who at age 77 is a professor emeritus at Caltech, the award is "a recognition of the power of pure genetics."

And other researchers think it would be hard to imagine better recipients. Their work "has had a huge impact on the field," says Richard Losick, a developmental biologist at Harvard University. "It's made it possible to understand how you get from a fertilized egg to a multicellular creature with specialized types of cells." Adds developmental geneticist Wolfgang Driever of Massachusetts General Hospital in Boston, "If you went through a modern textbook in developmental genetics and took out all the pages that couldn't have been written if these three people hadn't been around, there wouldn't be much left."

Subsequent work by the Nobelists and others has shown that genes similar to the ones that determine body pattern in *Drosophila* are at work in vertebrates, including mammals, and even in plants. Not only the genes and their protein products but also the sequence of their interactions "seem to be highly conserved," says David Hogness, a developmental biologist at Stanford University. Because of this conservation, work that began in the fruit fly is even helping explain defects that could be responsible for some miscarriages and congenital malformations in humans, a connection noted by the Nobel Assembly in explaining their choice.


KARL MATHIS/AP


MIKE DERER/AP


THOMAS KIENZLE/AP

The fly prize. *(Clockwise from top)* Edward B. Lewis, Christiane Nüsslein-Volhard, and Eric Wieschaus unraveled the genetics of *Drosophila* development.

The three winners, although of different generations, share a combination of courage and endurance. Lewis chose early in his career to study an odd class of fruit fly mutations in which entire body parts, such as an extra pair of wings, appear in unexpected locations. Called "homeotic" mutations (from the Greek for "likeness"), such changes were ascribed by many molecular biologists to simple coding errors within a single gene-protein system. But Cambridge University geneticist Michael Ashburner says that "Ed had the insight, right from the 1940s, that the homeotic genes were not just a curiosity—that their organization would be deeply interesting in a biological sense."

Lewis realized that in flies with an extra pair of wings an entire segment of the thorax had been omitted and replaced by a duplicate of the segment just in front of it. Over decades, Lewis collected and crossbred flies with other mutations that altered segment identities, classifying the abnormalities and mapping the locations of the affected genes along the fly's third chromosome. In this manner, he identified a series of control genes (later named "homeotic selector genes") that seemed to regulate the activity of other genes, eventually guiding the development of specialized features within each body segment (*Nature*, 7 December 1978, p. 565). Lewis also demonstrated the "colinearity principle": that homeotic selector genes appear on the chromosome in an order corresponding to the order of the body segments they influence (see diagram). This arrangement is possibly maintained because the regulatory regions that turn these genes on and off overlap. (The homeobox, a section of DNA that helps specify cell fate, was first detected in homeotic selector genes.)

But Lewis's work didn't explain crucial events upstream from this selector gene activity: the genetic changes that divide the embryo into primordial segments and lead to selector-gene activation in the first place. That question intrigued Nüsslein-Volhard and Wieschaus, who, in the late 1970s, were young group leaders at the European Molecular Biology Laboratory (EMBL) in Heidelberg, Germany. The two decided to embark on an ambitious "saturation screen" designed to detect all the genes affecting segmentation.

Sitting at a special dual microscope, Nüsslein-Volhard and Wieschaus spent a year scanning thousands of dead *Drosophila*

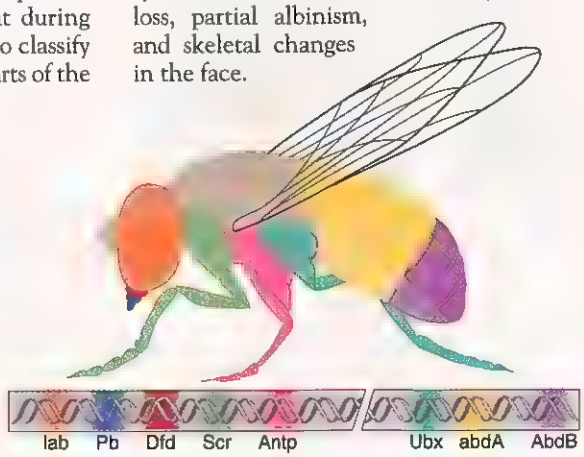
embryos—the second-generation offspring of flies exposed to mutagenic chemicals—for signs of defects in cuticle formation that would indicate abnormal segmentation. Explains Wieschaus, "We would ask, do the embryos of a given stock look abnormal in the same way? Is there a mutant phenotype that did something constant during development? Then we would try to classify the defects." Determining which parts of the embryo were deleted by lethal mutations, they hoped, would indicate how the affected genes normally function.

The project was a risky one, both scientifically and professionally. The screen could have turned up too many segmentation genes to fit into a meaningful classification scheme. And "in the context of a molecular biology lab, it was seen as a little weird" for the two scientists to restrict themselves entirely to genetic techniques, recounts Hermann Steller, a developmental neuroscientist who joined EMBL as a graduate student in 1981 and is now at the Massachusetts Institute of Technology.

But the saturation screen was "a phenomenally well-organized frontal assault on a problem, and relatively shortly into it they already knew they'd hit the jackpot," says Mark Peifer, a developmental biologist at the University of North Carolina, Chapel Hill, and a former postdoc in Wieschaus's lab at Princeton. That jackpot showed up when Nüsslein-Volhard and Wieschaus saw that their mutant flies fit into three distinct categories—which they named "gap," "pair-rule," and "segment polarity"—and proposed that three corresponding sets of genes act at different levels to progressively subdivide the embryo into segments.

According to a model that gained widespread acceptance shortly after the pair's landmark 1980 paper (*Nature*, 30 October 1980, p. 795), differing concentrations of a maternal gene product first activate the gap genes, dividing the embryo into broad regions. Later, the pair-rule genes subdivide these regions into segments, and finally, the segment polarity genes set up repeating anterior-to-posterior structures in each segment. The homeotic genes, identified by Lewis, are turned on in bands defined by the gap genes, and their action is refined by the pair-rule and segment-polarity genes.

"Untold thousands" of researchers are now analyzing the genes and gene families Lewis, Nüsslein-Volhard, and Wieschaus defined in *Drosophila* and their homologs in mice, chickens, zebrafish, humans, and other organisms, Peifer notes (*Science*, 13 May 1994, p. 904). The chicken gene *Sonic hedgehog*, a relative of the fruit fly segment-polarity gene *hedgehog*, for example, has recently been shown to play an important role in determining left-right asymmetry in the early embryo (*Cell*, vol. 82, p. 803, 1995). And mutations in a human gene related to the fruit fly pair-rule gene *paired* can cause Waardenburg's syndrome, a rare disease involving hearing loss, partial albinism, and skeletal changes in the face.

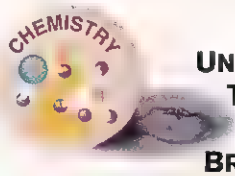


Prize-worthy parallels. Body segments in the fruit fly get their identities from a series of control genes that appear in the same order on the fly's chromosome.

SOURCE: S. GILBERT, *DEVELOPMENTAL BIOLOGY*, SINAUER ASSOC., 1994 ILLUSTRATION: K. SUTLIFF

"This Nobel shows that one door opened by a few original people can do more than a huge number of scientists have done before them," says Peter Lawrence, a *Drosophila* geneticist at the Laboratory of Molecular Biology in Cambridge, England. And once that door is opened, an equally large number of researchers can walk through.

–Wade Roush



UNCOVERING THREATS TO THE OZONE LAYER BRINGS REWARDS

Few scientific discoveries make a splash that ripples far beyond a specialized field, or beyond science in general. But the trio of atmospheric chemists who share this year's Nobel Prize in chemistry were honored for work that triggered scientific and political waves that continue to stir the waters even today. Their research identified chemicals that destroy stratospheric ozone, which shields Earth's plants and animals from harmful ultraviolet radiation. The discovery paved the way for an international agreement to ban the production of ozone-destroying compounds, known as chlorofluorocarbons (CFCs), beginning the first day of 1996.

"[The work] changed the complexion of what controls the global ozone distribution profoundly," says James Anderson, a professor of atmospheric chemistry at Harvard University in Cambridge, Massachusetts. Paul Crutzen, who works at the Max Planck Institute for Chemistry in Mainz, Germany, earned his share of the prize, in part, for his 1970 discovery that a naturally produced compound known as nitrous oxide makes its way to the stratosphere, where it spawns related chemicals that chew up individual ozone molecules. Atmospheric chemists F. Sherwood Rowland of the University of California, Irvine, and Mario Molina of the Massachusetts Institute of Technology won their Nobels for demonstrating in 1974 that industrially produced CFCs also drift up into the stratosphere, where they give rise to ozone-destroying reactions.

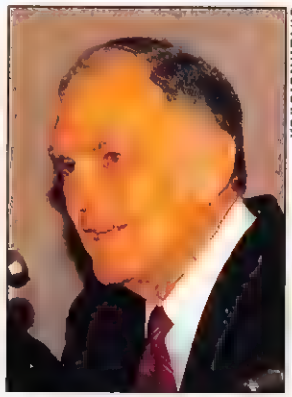
By detailing the fragile balance that maintains the ozone layer and showing how activity on Earth was perturbing it, "the three researchers contributed to our salvation from a global environmental problem that could have catastrophic consequences," says a statement from The Royal Swedish Academy of Sciences.

Ozone, a cluster of three oxygen atoms, is concentrated in a layer between 10 and 50 kilometers up. English physicist Sidney Chapman first explained the existence of this layer in 1930 by proposing that the energy from ultraviolet (UV) light converts various forms of oxygen—including atomic oxygen (O), molecular oxygen (O_2), and ozone (O_3)—from one to another, maintaining an equilibrium concentration of ozone. Chapman's mechanism turned out to be correct. But measurements made in the 1950s indicated that ozone concentrations were lower than they should have been. Something other than UV light had to be doing ozone in, researchers concluded, but the culprit remained a mystery.

In 1970, Crutzen pointed to a strong suspect: nitrogen oxides. He was tipped off by research showing that soil bacteria churn out a nonreactive oxide, nitrous oxide (N_2O). In a paper published that year in the *Quarterly Journal of the Royal Meteorological Society*, he outlined a chemical pathway that could transform this ground-produced substance into a high-altitude ozone-eater. He showed that nonreactive N_2O produced by soil bacteria could drift upwards into the stratosphere, where it would be broken apart by sunlight into two reactive nitrogen oxide compounds, NO and NO_2. He then suggested these nitrogen oxides convert ozone molecules into O_2 through a three-step reaction. And because NO and NO_2 are not consumed in the reaction, they continue to break down ozone until they eventually settle out of the atmosphere. By the mid-1970s, atmospheric measurements confirmed Crutzen's conclusions.

It didn't take long for these findings to have a political effect. In 1971, Crutzen's theory was cited by opponents of a U.S. program to build a fleet of supersonic transport (SST) aircraft. The planes were thought to


MANUEL DE LA FUENTE/AFP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The high prize. *(From left)* Paul Crutzen identified ozone-destroying reactions in the stratosphere. F. Sherwood Rowland and Mario Molina found some chemicals responsible were humanmade.

pose a grave threat to the ozone layer, as their exhaust would deliver nitrogen oxides right to its heart. (After much debate, the U.S. SST program was abandoned, largely due to fears of noise pollution and high costs.)

The political impact of Crutzen's work foreshadowed the reaction when, in 1974, Molina and Rowland published a paper in the 28 June issue of *Nature* suggesting that humans as well as bacteria were responsible for triggering ozone destruction. Ubiquitous industrial chemicals—chlorine-packed CFCs—traveled a path similar to that taken by the nitrous oxide, and with similar results. "We knew that CFCs would have a long lifetime," as they are chemically very stable, says Rowland. "So we started out to track CFCs from the cradle to the grave. The grave turned out to be the stratosphere." Here, it turned out, CFCs were broken down by UV light, liberating ozone-killing atomic chlorine (Cl) and chlorine monoxide (ClO). "That changed the entire context of global toxicology and showed that what we do on the surface of the Earth could affect the life-support system of the planet," says Anderson.

That stark fact was brought home in 1985, when a team of British researchers discovered the Antarctic ozone hole (see p. 376). CFCs turned out to play a major role in its formation, because they are carried over the pole by global air circulation. Molina went on to help determine that rapid destruction of ozone over the southern pole was largely due to the interaction between tiny ice particles and CFC byproducts such as chlorine nitrate and hydrochloric acid; the interaction transforms them into their more reactive cousins.

Their discoveries also fired up intense political heat to phase out CFCs, a battle that continues to rage today. Last month, for example, the U.S. Congress held hearings on whether the upcoming ban on CFC production should be postponed. Some partisans in this battle even see the Nobel selection as an effort to stave off such a move. "I think the Swedish Academy has chosen to make a political statement," says atmospheric chemist Fred Singer, who has long opposed calls for an early phaseout of CFCs.

But few other researchers see any hint of politics behind the choice of Crutzen, Molina, and Rowland. "I think it's an excellent selection," says Richard Stolarski, an atmospheric scientist at the National Aeronautics and Space Administration's Goddard Space Flight Center in Greenbelt, Maryland. "All three of them did things which formed a turning point in our understanding of the way the atmosphere worked."

–Robert F. Service

AN HONOR FOR CHAMPIONING RATIONAL THOUGHT

Erroneous assumptions lead to bad conclusions in any branch of science, but in economics they can lead to disastrous national policies. Many economists believe that's exactly what happened in the 1970s, when governments inflated monetary supplies to create jobs. The bad assumption? That in response to an increased supply of money, consumers would spend more and businesses would take on more workers. Instead, the result was "stagflation"—stagnant economic growth coupled with rapid inflation.


CHARLES BENNETT/AP

Rational reward. For demonstrating that individuals respond sensibly to policy changes, Robert J. Lucas won the economics award.

In the mid-1970s, University of Chicago economist Robert E. Lucas Jr. developed a theory that explained why the assumption was wrong. Individuals, he argued, respond to inflation—an erosion of their buying power—by demanding higher wages, not by spending more; businesses recognize that inflated prices don't indicate increased demand and so do not hire more workers to expand production. These common-sense responses were part of Lucas's theory of "rational expectations," a view of macroeconomics that has garnered Lucas this year's Nobel Memorial Prize in Economic Sciences. In its award citation, the Royal Swedish Academy of Sciences described him as "the economist who has had the greatest influence on macroeconomic research since 1970."

Colleagues agree. "I consider him the economist of his generation, maybe of this half-century," says Edward C. Prescott, professor of economics at the University of Minnesota. "He has had a remarkable influence on economic science," he adds. Lucas's Chicago colleagues are equally fulsome in their praise—"very well-deserved" was the assessment of José Scheinkman, chair of the school's economics department—although praising Nobelists is getting to be routine for Chicago economists. The school's faculty members have won the economics prize in 5 of the last 6 years and eight times since 1976. "I was afraid [the Swedish Academy] might shy away from picking someone at Chicago again so soon," says Lars Hansen, another Chicago economist. "I thought they might make Lucas wait a bit longer." Fortunately for Lucas, that expectation was wrong.

Expectations—erroneous and accurate—are at the heart of Lucas's theory. Until the 1970s, economic policy-makers generally thought they could ignore expectations in their attempts to fine-tune national economies, especially in their embrace of the Phillips curve, an equation implying that jobs could be created through an inflationary monetary policy.

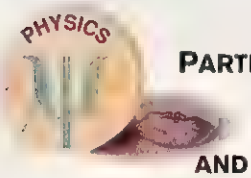
Lucas believed that such an expectation wasn't rational. It "assumed a lot of stupidity on the part of the ordinary citizen," he explained at a press conference in Chicago last week, when the award was announced. His studies demonstrated that workers' rational response—to demand higher wages—would soak up business capital that monetary gurus thought would be used to hire new employees. The result: price inflation without real economic growth. And that was exactly what many economies suffered through the 1970s. "[Lucas] explained the failure," Scheinkman says.

While his analysis of policy-making may be the most visible aspect of Lucas's work,

Prescott notes that his influence extends throughout economics. He says Lucas took analytical principles that had been used only in the highly theoretical field of microeconomics and showed how they could be used to solve practical macroeconomic problems. "He has unified economics," Prescott says.

Hansen believes that this blurring of the boundaries of economic subfields is one of the distinguishing characteristics of the atmosphere at Chicago, and something that might be contributing to the school's domination of recent economics Nobels. "Distinctions between fields don't mean much here," he says, adding that everyone is willing to critique the work of colleagues even if it is outside their area of interest. And Lucas doesn't expect any deference on account of his award. "Around here a Nobel Prize doesn't carry much weight in an argument," he said at his press conference. "Around here you've got to win an argument on its merit."

–Dennis Normile



PARTICLE HUNTERS BAG TWO TROPHIES—AND SHARE A THIRD

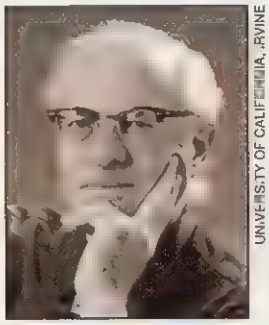
Martin Perl and Frederick Reines never collaborated. Their experimental techniques were worlds apart, and their achievements, which brought them this year's Nobel Prize in physics, have only two things in common, as far as their colleagues can tell: Both discovered new members of a class of fundamental particles of matter known as leptons (the other basic group is made of quarks), and both prizes are long overdue.

Reines, a professor emeritus of physics at the University of California (UC), Irvine, won his share of the prize for detecting the apparently massless particle called the neutrino. He pulled off this feat in 1956, working with the late Clyde Cowan at the Los Alamos National Laboratory. Perl, of Stanford University and the Stanford Linear Accelerator Center (SLAC), was honored for his role in the discovery of the tau lepton, a close relative of the electron albeit 3500 times heavier. With collaborators at SLAC, Perl pursued his quarry between 1974 and 1977. The discoveries of almost massless and extremely massive leptons set the stage for our current understanding of the relationships among elementary particles, which are divided into three families of two leptons and two quarks each.

As far as the physics community was concerned, those achievements were Nobel-worthy years ago. Columbia University's Mel Schwartz, who shared the 1988 Physics Prize, called it "a fabulous choice," and added "for years, I've been thinking those guys really deserve it." UC Irvine's Henry Sobel, who has studied and worked with Reines on neutrino physics since 1963, said simply, "Better late than never."

Reines's discovery of the neutrino was all the more remarkable because of its elusive nature. Wolfgang Pauli, who first proposed the existence of the particle back in 1930, believed he had done a "frightful" thing by postulating an entity that would likely never be detected. Pauli had invoked the idea to account for the infinitesimal energy and momentum that seemed to be missing in certain radioactive decays of atoms. But the particle has no charge and no apparent mass; it rarely interacts with matter at all, slipping through virtually any detector without a trace.

In 1953, however, Reines and Cowan proposed an ingenious neutrino trap: a 120-gallon tank of water salted with cadmium atoms, surrounded by light detectors. The apparatus would sit next to a nuclear reactor, which theoretically should be emitting hundreds of trillions of neutrinos a second. The goal was to detect flashes of light emitted in the extremely rare instances when a neutrino hit a hydrogen nucleus. Such an interaction, they reasoned, would create two particles, a positron and a neutron. The positron would almost immediately hit an electron, releasing two low-energy photons. But the neutron would take a slower route, plunging through the water until it was captured by a cadmium nucleus; the capture would release high-energy gamma rays. The time delay—a few microseconds—between the photon and the gamma ray releases would be the neutrino's signature. Reines and Cowan were able to identify a few neutrinos each hour. As the Nobel citation put it: "They had raised the neutrino from its status as a figure of the imagination to an existence as a free particle."


UNIVERSITY OF CALIFORNIA, IRVINE


PAUL SAKUMA/AP

Odd couple. Frederick Reines *(top)* found an apparently massless elementary particle of matter, while Martin Perl *(bottom)* found one of the heavier leptons.

Reines went on to pioneer the field of underground physics, building neutrino detectors in the bottom of mine shafts to avoid interference from cosmic radiation, as well as to help kick-start the field of neutrino astronomy. Says Ken Lande, a neutrino physicist at the University of Pennsylvania in Philadelphia: "He has always had novel ideas, novel approaches to doing physics, totally out of the conventional domain."

While Reines worked in small collaborations, Perl did the bulk of his path-breaking physics as a member of the first large American physics collaboration at SLAC. He joined SLAC in 1963, hoping to find heavier versions of the electron, one of which, the muon, was already known. Throughout the sixties, Perl searched through the debris of particle collisions at SLAC for still heavier versions of the electron. But as he puts it, he "couldn't get any leads." Then SLAC started building an electron-positron collider, SPEAR, which operated in a previously inaccessible energy realm, making the detection of a more massive electron possible. Perl and his group hoped to find collisions that produced pairs of muons and electrons together—a combination that could only come from the annihilation of some new heavier lepton in the collision.

Although Perl identified candidate events in the earliest data from SPEAR, he says "nobody believed it at first even at SLAC." It took him months to convince his own collaborators. Finally by late 1974, the group published a paper announcing the discovery, first called the "U" particle for "unknown."

For the next 3 years, says Perl, he had a lot of "sleepless nights" as the physics community seemed more intent on proving him wrong than proving him right. "People kept not finding it," he says. Not until 1977 did Perl finally hear that tau particles had been found at other accelerators and that his discovery had been confirmed.

The discovery of the tau, explains Burton Richter, SLAC's director, "was a complete surprise to physics." By the time it was made, physicists had realized that the existing elementary particle zoo could be divvied up into two families, each with two quarks and two leptons. The lightest family had up and down quarks, and for leptons an electron and an electron neutrino. The heavier family consisted of charm and strange quarks, together with a muon and a muon neutrino. The tau meant there had to be yet a third generation.

As for Perl, he says the Nobel Prize also completes another triad. He got his Ph.D. at Columbia as a student of I. I. Rabi, who won the Nobel Prize in 1944, and his students included Sam C.C. Ting, who shared the prize with Richter in 1976. "So there are three generations, too," says Perl.

–Gary Taubes

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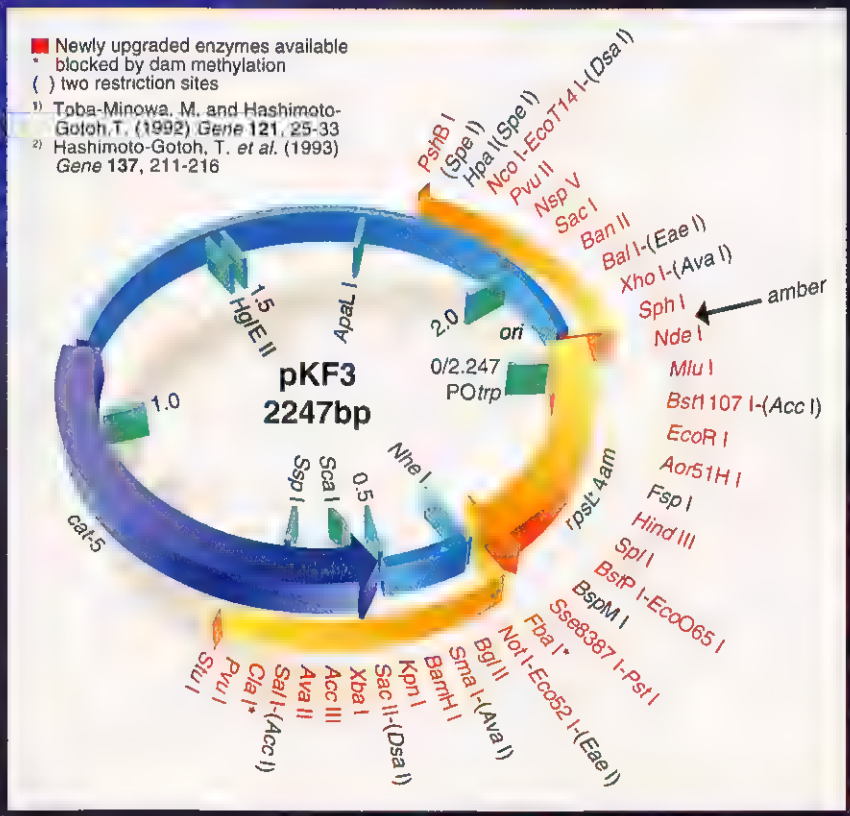

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Fax: +81 775-43-9254

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Phone: (1) 41 47 01 14
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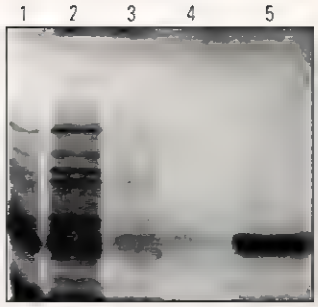
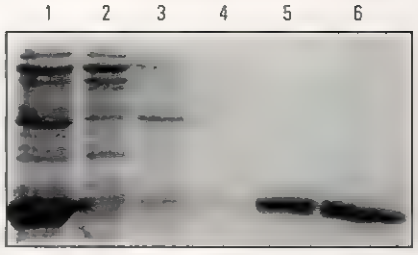


Native. N-terminal, 6xHis mouse dihydrofolate reductase (DHFR, 19.5 kDa) was expressed in *E. coli*. 800 µl of the clarified lysate was applied to 100 µl of TALON. All bound protein was eluted with 300 µl of 100 mM EDTA, pH 8.0. 20 µl of lysate and 40 µl of each subsequent fraction was loaded onto a 12% polyacrylamide/ SDS gel. Lane 1: clarified lysate. Lane 2: flowthrough. Lane 3: first wash. Lane 4. third wash. Lane 5. DHFR final elution.



Denaturing N-terminal, 6xHis mouse DHFR (19.5 kDa) was expressed in *E. coli* 600 µl of clarified lysate was applied to a TALONspin Column containing 0.5 ml of TALON-NX Metal Affinity Resin and centrifuged for 2 min at 2,000 x g. 20 µl of lysate (Lane 1) and 40 µl of each subsequent fraction was loaded onto a 12% polyacrylamide/SDS gel. Lane 1: clarified lysate. Lane 2. flowthrough. Lane 3: first pH 7.0 wash. Lane 4: second pH 7.0 wash. Lane 5: DHFR, first pH 6.0 elution. Lane 6: DHFR, second pH 6.0 elution.

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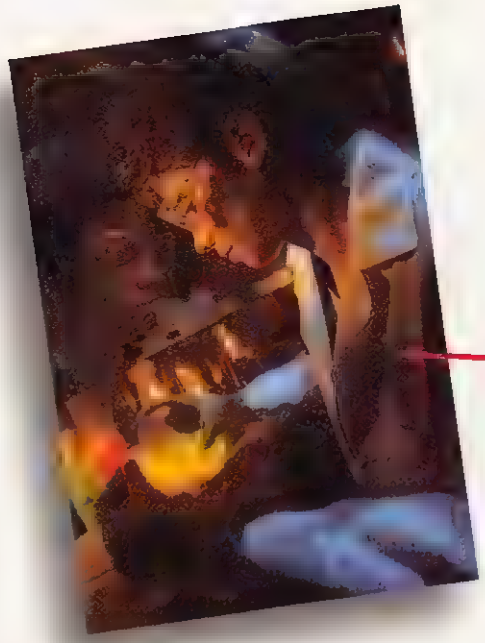

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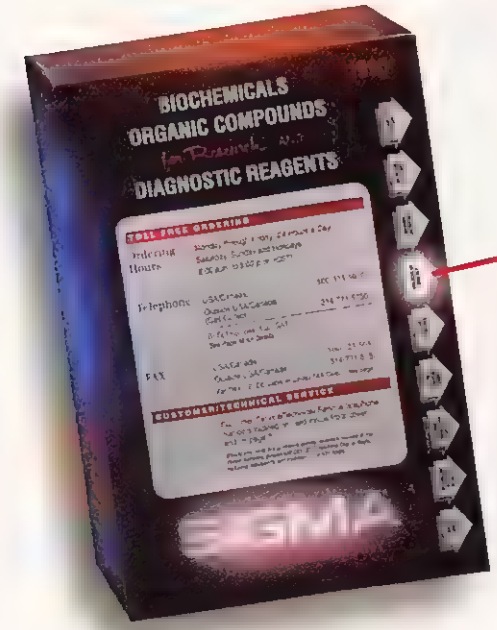

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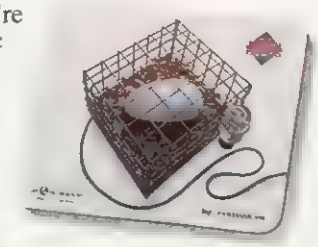

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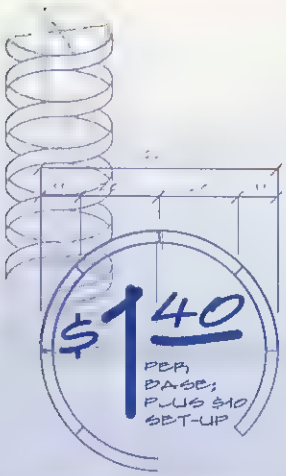

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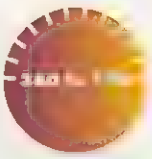
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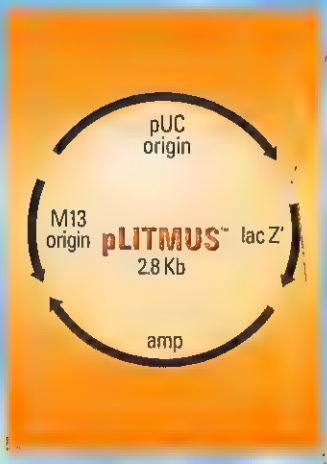

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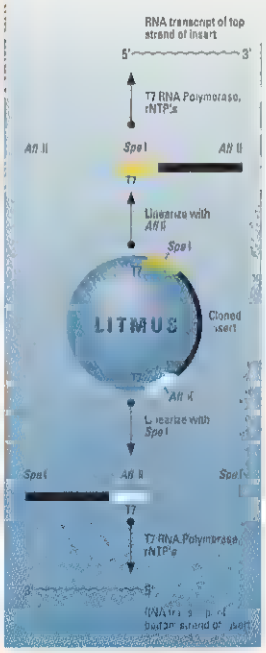

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Genetic Discrimination and Health Insurance: An Urgent Need for Reform

Kathy L. Hudson, Karen H. Rothenberg, Lori B. Andrews, Mary Jo Ellis Kahn, Francis S. Collins

The accelerated pace of gene discovery and molecular medicine portend a future in which information about a plethora of disease genes can be readily obtained. As at-risk populations are identified, research can be done to determine effective prevention and treatment strategies that will lower the personal, social, and perhaps the financial costs of disease in the future. We all carry genes that predispose to common illnesses. In many circumstances knowing this information can be beneficial, as it allows individualized strategies to be designed to reduce the risk of illness. But, as knowledge about the genetic basis of common disorders grows, so does the potential for discrimination in health insurance coverage for an ever increasing number of Americans.

The use of genetic information to exclude high-risk people from health care by denying coverage or charging prohibitive rates will limit or nullify the anticipated benefits of genetic research. In addition to the real and potentially devastating consequences of being denied health insurance, the fear of discrimination has other undesirable effects. People may be unwilling to participate in research and to share information about their genetic status with their health care providers or family members because of concern about misuse of this information. As genetic research progresses, and preventive and treatment strategies are developed, it will be increasingly important that discrimination and the fear of discrimination not be a roadblock to reaping the benefits. To address these issues, the National Institutes of Health–Department of Energy (NIH-DOE) Working Group on Ethical, Legal, and Social Implications (ELSI) of the Human Genome Project and the National Action Plan on Breast Cancer have jointly developed a series of recommendations for state and federal policymakers which are presented below.

In the past, genetic information has been used by insurers to discriminate against people. In the early 1970s, some insurance companies denied coverage and charged higher rates to African Americans who were carriers of the gene for sickle cell anemia (*1*). Contemporary studies have documented cases of genetic discrimination against people who are healthy themselves but who have a gene that predisposes them or their children to a later illness such as Huntington's disease (*2*). In a recent survey of people with a known genetic condition in the family, 22% indicated that they had been refused health insurance coverage because of their genetic status, whether they were sick or not (*3*).

As a case example, Paul (not his real name) is a healthy, active 4-year-old, but he has been twice denied health insurance. Paul's mother died in her sleep of sudden cardiac arrest when Paul was only 5 months old. Paul's maternal uncle also died of sudden cardiac arrest when he was in his twenties. After these sudden and unexpected deaths, Paul's family began a hunt to discover the cause. Their search finally led to a research geneticist who was able to determine that several family members, including Paul and his mother, carried an alteration in a gene on chromosome 7. This gene is one of several genes that causes the long QT syndrome, so-called because of the distinctive diagnostic pattern on an electrocardiogram.

Several years ago, Paul's father, Bob, lost his job and with it the group policy that provided health insurance coverage for Paul and him. Paul's father has repeatedly applied for a family health insurance policy with a major insurance company. The company agreed to cover Bob but refused to issue a family policy that would cover Paul because he has inherited the altered gene for the long QT syndrome from his mother.

The story of Jackie and Emma further illustrates the social, ethical, and legal dilemmas presented by the revelation of genetic information. Sisters Jackie and Emma, along with many other members of their family, have been tested as part of a research protocol for alterations in the gene, *BRCA1*, that confers hereditary susceptibility to breast and ovarian cancer. Both were offered an opportunity to learn the results of their genetic tests and both accepted. They each learned they carry an altered form of the gene, putting them at increased risk for breast and ovarian cancer.

After finding out the results of her genetic test, Emma had a mammogram that showed a very small lesion in her breast. A subsequent biopsy revealed carcinoma, and Emma decided to proceed with a bilateral mastectomy because of the substantial risk of cancer arising in the opposite breast. Her lymph nodes were negative for cancer, so her prognosis for cure is very good.

Emma's sister Jackie also tested positive for the same alteration in the *BRCA1* gene, though no cancer was detected. Although the benefit of prophylactic mastectomy in reducing the risk for breast cancer is not yet known, she decided to have a bilateral prophylactic mastectomy. Emma and Jackie feel strongly that they have benefited from knowing this genetic information but are fearful that it will be used against them and their family by insurers and employers. They both keep their genetic status secret and are so fearful of losing their health insurance that they used assumed names when sharing their story at a recent workshop on genetic discrimination (*4*).

Emma and Jackie's story is not unique. An estimated 1 in 500 women carry a mutation in the *BRCA1* gene that may confer as much as an 85% chance of breast cancer and a 50% chance of ovarian cancer (*5*). Although substantial uncertainty exists about the relative value of the available options (surgery compared with intensive surveillance) for a woman with a *BRCA1* mutation, it is likely that ultimately this information will be medically useful.

Health Insurance in the United States

Because of high costs, insurance is essentially required to have access to health care in the United States. Over 40 million people in the United States are uninsured (*6*). Group insurance, individual insurance, self-insurance, and publicly financed insurance (for example, Medicare and Medicaid) are the principal forms of health insurance in the United States for the ~240 million Americans with coverage. Most people get their health insurance through their employer. Many employers provide health insurance coverage through self-funded plans in which the employer, either directly or through a third party, provides health insurance coverage. For individuals and small groups, insurance providers use medical history as well as individual risk factors, such as smoking, to determine whether to provide coverage and under what terms. This is

K. L. Hudson is assistant director for Policy Coordination, National Center for Human Genome Research, National Institutes of Health (NIH). K. H. Rothenberg is Marjorie Cook Professor of Law and director of the Law and Health Care Program, University of Maryland School of Law, and member of the National Action Plan on Breast Cancer (NAPBC). L. B. Andrews is chair of the NIH–Department of Energy Working Group on Ethical, Legal, and Social Implications and professor at Chicago-Kent College of Law. M. J. Ellis Kahn represents the Virginia Breast Cancer Foundation and the National Breast Cancer Coalition and is co-chair of the Hereditary Susceptibility Working Group, NAPBC. F. S. Collins is director of the National Center for Human Genome Research, NIH, and co-chair of the Hereditary Susceptibility Working Group, NAPBC.

known as underwriting. Insurers argue that underwriting is essential in a voluntary market to prevent "adverse selection," in which individuals elect not to purchase insurance until they are already ill or anticipate a future need for health care. Insurers fear that individuals will remain uninsured until, for example, they receive a genetic test result indicating a predisposition to some disease such as breast or colon cancer.

In the absence of the ability to detect hereditary susceptibility to disease, the costs of medical treatment have been absorbed under the current health insurance system of shared risk and shared costs. Today, our understanding of the relation between a misspelling in a gene and future health is still incomplete, thus limiting the ability of insurers to incorporate genetic risks into actuarial calculations on a large scale. As genetic research enhances the ability to predict individuals' future risk of diseases, many Americans may become uninsurable on the basis of genetic information.

State and Federal Initiatives

A recent survey has shown that a number of states have enacted laws to protect individuals from being denied health insurance on the basis of genetic information (Fig. 1) (*7*). The first laws addressing genetic discrimination were quite limited in scope and focused exclusively on discrimination against people with a single genetic trait such as sickle cell trait (*8*). Since the Human Genome Project was launched in 1990, eight states have enacted some form of protection against genetic discrimination in health insurance. The recently enacted state laws are not limited to a specific genetic trait but apply potentially to an unlimited number of genetic conditions. These state laws prohibit insurers from denying coverage on the basis of genetic test results, and prohibit the use of this information to establish premiums, charge differential rates, or limit benefits. A few of these states, including Oregon and California, integrate protection against discrimination in insurance practices with privacy protections that prohibit insurers from requesting genetic information and from disclosing genetic information without authorization.

Two factors limit the protection against discrimination afforded by current state laws. First, the federal Employee Retirement Income Security Act exempts self-funded plans from state insurance laws. Nationwide, over one-third of the nonelderly insured population obtains health insurance coverage through a self-funded plan. Second, nearly all of the state laws focus narrowly on genetic tests, rather than more broadly on genetic information generated by family history, physical examination, or the medical record (*7*). Limiting the scope of protection to results of genetic tests means that insurers are only prohibited from using the results of a chemical test of DNA, or in some cases, the protein product of a gene. But insurers can use other phenotypic indicators, patterns of inheritance of genetic characteristic, or even requests for genetic testing as the basis of discrimination. Meaningful protection against genetic discrimination requires that insurers be prohibited from using all information about genes, gene products, or inherited characteristics to deny or limit health insurance coverage.

No federal laws are currently in place to prohibit genetic discrimination in health insurance (*9*). The Clinton Administration's proposal to reform the health care system and provide health insurance for all Americans did prohibit limiting access or coverage on the basis of "existing medical conditions or genetic predisposition to medical conditions" (*10*). Congressional efforts to reform the health care system in 1995 have been much more modest and are targeted at guaranteeing access, portability, and renewability of coverage and at leveling the playing field in the insurance market so that the same rules apply to insured and self-funded plans. Recent federal health insurance reform proposals attempt to guarantee the availability of health care by prohibiting insurers from denying coverage on the basis of health status, medical condition, claims experience, or medical history of a participant. Most of the proposals permit exclusions for pre-existing conditions, but these are time limited.

It is not clear if the current health insurance reform proposals would prohibit insurers from denying coverage on the basis of genetic information. Genetic information is distinct from other types of medical information because it provides information about an individual's predisposition to future disease. In addition, genetic information can provide clues to the future health risks for an individual's family members. If enacted, current health reform proposals would prohibit denying insurance to those currently suffering from disease or with a past history of disease. But these proposals may not protect people like Paul, who are healthy but have a genetic predisposition to disease, from being refused insurance coverage. Current proposals also may fail to protect couples who, although healthy themselves, carry the gene for a recessive disorder such as cystic fibrosis that might affect their children or future children.

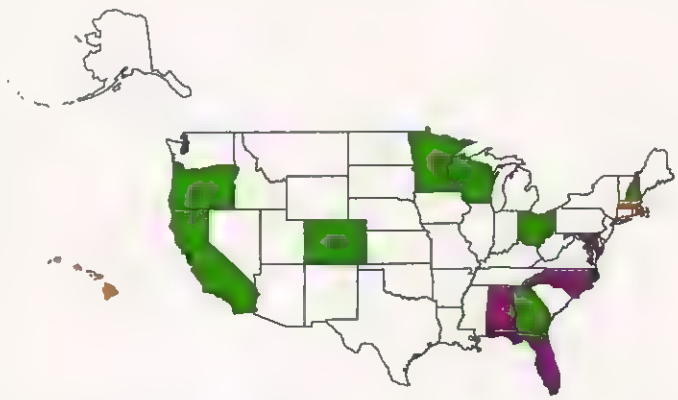

Fig. 1. State laws on the use of genetic information in health insurance (*7*). States shown in purple were the first states to enact legislation addressing genetic issues in insurance. Florida and Alabama laws prohibit insurers from denying coverage on the basis of the sickle cell trait. North Carolina prohibits insurers from denying coverage because the applicant has the hemoglobin C or sickle cell trait. Maryland prohibits discrimination in rates based on any genetic trait unless there is actuarial justification. States shown in green (California, Oregon, Colorado, Minnesota, Wisconsin, Ohio, Georgia, and New Hampshire) prohibit insurers, to varying degrees, from requiring or requesting genetic tests or their results, from denying coverage on the basis of genetic tests, and from using tests to determine rates and benefits. California, Colorado, Oregon, and Wisconsin laws include provisions to protect the privacy of genetic information. States shown in orange (Massachusetts and Hawaii) have related bills pending.

Recommendations

Planners of the Human Genome Project recognized from the beginning that maximizing the medical benefits of genome research would require a social environment in which health care consumers were protected from discrimination and stigmatization based on their genetic make-up. Genome programs at both the DOE and the National Center for Human Genome Research, a component of NIH, have each set aside a portion of their research budget to anticipate, analyze, and address the ELSI of new advances in human genetics. The original planners also created the NIH-DOE ELSI Working Group, which has a broad and diverse membership including genome scientists; medical geneticists; experts in law, ethics, and philosophy; and consumers, to explore and propose options for the development of sound professional and public

policies related to human genome research and its applications. The ELSI Working Group has long been involved in discussions about the fair use of genetic information. In a 1993 report, "Genetic Information and Health Insurance" (*11*), the ELSI Working Group recommended a return to the risk-spreading goal of insurance. The Working Group suggested that individuals be given access to health care insurance irrespective of information, including genetic information about their past, current, or future health status. Because denial of insurance coverage for a costly disease such as breast cancer may prove to be a death sentence for many women, the National Action Plan on Breast Cancer (NAPBC), a public-private partnership designed to eradicate breast cancer as a threat to the lives of American women, has identified genetic discrimination in health insurance as a high priority (*12*).

Building on their shared concerns, the NAPBC (*13*) and the ELSI Working Group (*14*) recently cosponsored a workshop on genetic discrimination and health insurance (*4*). Scientists, representatives from the insurance industry, and members of the ELSI Working Group and the NAPBC participated in the 1-day session. On the basis of the information presented at the workshop, the ELSI Working Group and the NAPBC developed the following recommendations and definitions for state and federal policymakers to protect against genetic discrimination.

1) Insurance providers should be prohibited from using genetic information, or an individual's request for genetic services, to deny or limit any coverage or establish eligibility, continuation, enrollment, or contribution requirements.

2) Insurance providers should be prohibited from establishing differential rates or premium payments based on genetic information or an individual's request for genetic services.

3) Insurance providers should be prohibited from requesting or requiring collection or disclosure of genetic information.

4) Insurance providers and other holders of genetic information should be prohibited from releasing genetic information without prior written authorization of the individual. Written authorization should be required for each disclosure and include to whom the disclosure would be made.

The definitions are as follows. Genetic information is information about genes, gene products, or inherited characteristics that may derive from the individual or a family member. Insurance provider means an insurance company, employer, or any other entity providing a plan of health insurance or health benefits including group and individual health plans whether fully insured or self-funded.

These recommendations have been endorsed by the National Advisory Council for Human Genome Research (NACHGR) (*15*). The NACHGR stresses the positive value of genetic information for improving the medical care of individual patients and the need to ensure the freedom of patients and their health care providers to use genetic information for patient care. The NACHGR views the elimination of the use of genetic information to discriminate against individuals in their access to health insurance as a critical step toward these goals.

The ability to obtain sensitive genetic information about individuals, families, and even populations raises profound and troubling questions about who will have access to this information and how it will be used. The recommendations presented here for state and federal policy-makers are intended to help ensure that our current social, economic, and health care policies keep pace with both the opportunities and challenges that the new genetics present for understanding the causes of disease and developing new treatment and preventive strategies.

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13. NAPBC steering committee: Susan J. Blumenthal (co-chair), Zora Kramer Brown, Doris Browne, Anna K. Chacko, Francis S. Collins, Nancy W. Connell, Kay Dickersin, Arlyne Draper, Nancy Evans, Harmon Eyre, Leslie Ford, Janyce N. Hedetniemi, Mary Jo Ellis Kahn, Amy S. Langer, Susan M. Love, Alan Rabson, Jane Reese-Coulbourne, Irene M. Rich, Barbara K. Rimer, Susan Sieber, Edward Sondik, and Frances M. Visco (co-chair). NAPBC hereditary susceptibility working group: Kathleen A. Calzone, Francis S. Collins (co-chair), Sherman Elias, Linda Finney, Judy E. Garber, Ruthann M. Giusti, Jay R. Harris, Joseph K. Hurd Jr., Mary Jo Ellis Kahn (co-chair), Mary-Claire King, Caryn Lerman, Mary Jane Massie, Paul G. McDonough, Patricia D. Murphy, Philip D. Noguchi, Barbara K. Rimer, Karen H. Rothenberg, Karen K. Steinberg, and Jill Stopfer.
14. ELSI working group: Betsy Anderson, Lori Andrews (chair), James Bowman (dissenting), David Cox, Troy Duster, (vice chair), Rebecca Eisenberg, Beth Fine, Neil Holtzman, Philip Kitcher, Joseph McInerney, Jeffrey Murray, Dorothy Nelkin, Rayna Rapp, Marsha Saxton, and Nancy Wexler.
15. NCHGR council members: Anita Allen, Lennette J. Benjamin; David Botstein, R. Daniel Camerini-Otero (dissents with recommendation 3), Ellen W. Clayton, Troy Duster, Leroy E. Hood, David E. Housman, Richard M. Myers, Rodney Rothstein, Diane C. Smith, Lloyd M. Smith, M. Anne Spence, Shirley M. Tilgham, and David Valle.

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A Time to Sequence

Maynard V. Olson

My views on the Human Genome Project first appeared in *Science* in 1987 in the form of a one-word quotation: "Huge" (*1*). Eight years later, in deference to the size and complexity of the current program, I have been allotted more space. I use it here to examine the state of the project and to suggest a path forward.

The basic game plan for an organized Human Genome Project in the United States was established by a National Research Council committee, chaired by Bruce Alberts (*2*). Indeed, I was testifying before this committee in 1987 when my assessment of the project's scale caught a *Science* reporter's attention. The Alberts Committee recommended an early emphasis on genetic linkage mapping and clone-based physical mapping of human DNA. In parallel, the committee recommended research on the technology of DNA sequencing, as well as pilot-scale sequencing of the genomes of model organisms. This approach was viewed as the best way to improve the reliability of DNA sequencing—and to drive down its cost—while simultaneously gathering data of immediate biological value.

To a remarkable degree, the Alberts Committee read the historical and technical trends correctly. It now appears that even its estimates of time scale and cost—15 years at $200 million per year—were about right. In 1987, skeptics could still argue about basic feasibility with some force. Conversely, many of the project's proponents lacked a realistic sense of the diversity of problems that had to be solved before mammalian-scale sequencing would become practical. Even the Alberts Committee's middle-of-the-road recommendations would likely have proven to be overambitious if it were not for several unforeseen developments. Technically, the most important of these has been the emergence of the polymerase chain reaction (PCR) as a primary tool for DNA analysis. Rapid advances in computer technology have also been significant, particularly because they have allowed most of the project's data-analysis and data-management needs to be met by the distributed efforts of small groups of programmers working in close collaboration with experimentalists. Finally, vigorous international participation in the project has materialized, a development that the Alberts Committee strongly encouraged but could not count on.

The policy success of defining and implementing a program of this complexity in the face of rapidly evolving technology—and on a relatively austere budget—provides grounds for satisfaction (*3*). Nonetheless, the project's greatest challenge lies ahead. The preliminary phase of the Human Genome Project emphasized diverse lines of research, many of which could be pursued in conventional molecular biology laboratories. Much of this activity must ultimately be displaced by a more monolithic sequencing program, largely focused on human DNA. Neither the Alberts Committee nor subsequent policy reviews (*4*) provide clear guidance on how or when to carry out this transition. Recently, proponents of an early and aggressive move to very large-scale sequencing of human DNA have emerged from among the leaders of model-organism sequencing initiatives (*5*). In this Policy Forum, I add my support to their proposal. The case in favor of an early transition to human sequencing rests on an assessment of three questions: Are the maps good enough? Is the technology strong enough? and Would it be good policy?

The Maps

Almost certainly, the maps are good enough. This assessment rests on the current state of the maps, the rate at which they are improving, and the advantages of combining the last stages of physical mapping with sequencing. The dominant low-resolution mapping paradigm is sequence-tagged site (STS)–content mapping, applied either to comprehensive yeast artificial chromosome (YAC) libraries (*6*) or to panels of human-rodent hybrid cell lines that contain multiple segments of human DNA [that is, "radiation-hybrid," or RH, cell lines (*7*)]. These forms of mapping define the order of STSs, which are short, unique DNA sequences most commonly detected by PCR assays (*8*). STS ordering is inferred from data on the STS content (that is, presence or absence of particular STSs) in the random segments of the human genome present in a set of clones that has been organized into a "typing resource." The ability of a typing resource to resolve the order of STSs is determined by the average spacing between segment ends, typically 50 to 100 kbp in current resources. Maps with an average spacing between STSs of approximately 100 kbp already exist for perhaps 15% of the genome. Approximately half the genome has been mapped only by whole-genome approaches that thus far have produced average spacings closer to 300 kbp. The balance of the genome is at an intermediate state. There are also regions that have progressed beyond, or even bypassed, the STS-mapping stage, but they constitute only a small fraction of the total.

Because efficient screening methods exist for finding new clones that contain a particular STS (*9*), the choice of which clones to sequence at a particular site in the genome can be made immediately before the sequencing is carried out. There is presently healthy competition between cloning systems such as cosmids, P1-based clones, and bacterial artificial chromosomes (BACs), all of which provide plausible ways to clone the DNA that will actually be sequenced (*10*). The recombinant DNA molecules generated by these cloning systems contain 40 to 200 kbp of human DNA. Various "fingerprinting" and "contig-building" strategies allow contigs (that is, collections of overlapping clones that collectively cover the target region) to be built whose lengths are typically a few times the size of the clones from which they are constructed (*11*).

Because the spacings between mapped STSs are already comparable to the sizes of contigs that can be readily seeded around an STS, even current maps would allow much of the genome to be covered with well-mapped clones that are suitable for sequencing. Current mapping projects have enough momentum to reduce average STS spacings to 100 kbp throughout the genome within a year or two. Even with these maps, it is inevitable that there will be many clones sequenced whose precise genomic positions and left-right orientations cannot be determined simply from their STS content. However, it would be sensible to handle these cases by developing additional STSs at the ends of those sequenced clones whose positions and orientations are uncertain, rather than to continue random STS mapping to an unnecessarily high resolution throughout the genome. This strategy would answer the question: How good does the physical map need to be? with the most economical possible answer—just good enough to allow all sequence tracts to be aligned with it.

The resolution of the physical map required to support sequencing exceeds that needed to maintain alignment between the physical map, the genetic linkage map, and the cytogenetic map. Therefore, as the sequence of the human genome emerges, it will be possible to align sequence tracts with the genetic and cytogenetic maps, as well as the physical map, thereby allowing correlations between

The author is in the Department of Molecular Biotechnology, University of Washington, Seattle, WA 98195, USA.

particular sequences and observations on mutant human chromosomes.

The Technology

The question of whether or not sequencing technology is adequate for a near-term, massive increase in the scale of genomic sequencing is more troublesome than the corresponding question about maps. Many participants in the Human Genome Project, including this author, envisioned the project as a vehicle for developing powerful new sequencing tools that would displace the techniques of the 1980s through a combination of fundamental advances and automation.

What has happened instead is arguably a better development for experimental biology. Sequencing methodology has improved incrementally in a way that is leading to convergence, rather than divergence, between the methods employed in "genome centers" and those used in more typical molecular biology laboratories. Following a period of competition between alternative sequencing strategies, a dominant technology has emerged for large-scale genomic sequencing: Clones the size of cosmids or larger are analyzed by random sampling (that is, "shotgun" sequencing), implemented on commercial, four-color fluorescence sequencing instruments (*12*). The optimum size of the starting clones, the level of detail with which these clones should be mapped, and the extent to which random sampling should be supplemented by more "directed" methods remain contentious. However, the important news is that the basic approach works in any of several well-tested variations.

In retrospect, the idea that sequencing technology would be displaced in a few years by fundamentally new approaches was implausible. Major advances in analytical techniques are neither more frequent nor any easier to stimulate programmatically than are other scientific developments. Gel electrophoresis was first used to separate biological macromolecules on 23 January 1954 (*13*). Forty years later, it has changed relatively little while playing a key role in one revolutionary discovery after another in basic biology.

The envisioned transition to high-level automation of DNA sequencing was also unrealistic. The Human Genome Project lacks both the financial and human resources to bring it about. Clearly, contemporary sequencing procedures could be fully automated with a sufficient engineering investment. Less clear is how long it would take, what it would cost, and how competitive the result would be with more labor-intensive methods. The most realistic policy would be to continue to seek efficiency gains through the piecemeal introduction of labor-saving devices. As sequencing is implemented on a larger scale and cost containment becomes a paramount concern, it should become progressively easier to spot bottlenecks that could be overcome by specialized equipment.

An uncomfortable corollary to the emergence of a dominant technology is that it is time to curtail support for competing approaches. Small-scale exploration of genuinely novel approaches remains appropriate. However, it is time to recognize that genomic sequencing is in the coalescence phase of the alternating periods of competition and coalescence by which complex technologies lurch from one generation to the next. During this phase, the dominant technology improves rapidly and declines in cost just because it is dominant.

Policy Implications

Even if the maps and technology are judged adequate, there remains a question as to whether or not it is a good idea to divert resources from other activities to large-scale human sequencing. Program areas that would be adversely affected, together with brief arguments supporting their importance, are summarized below.

Technology. Further technological development would reduce the cost of human sequencing and allow the sequencing of other genomes. Overinvestment in sequencing capacity on the basis of current technology would suppress innovation and create large facilities that would rapidly become obsolete.

Informatics. Data collection is outstripping current capabilities to annotate, store, retrieve, and analyze maps and sequences. Better computational tools will be necessary before biologists will be able to make effective use of the data.

Disease. An important motivation for the Human Genome Project is to make it easier to analyze human genetic diseases. Activities such as intensive mapping of expressed-sequence tags and light sampling of genomic sequence provide the cheapest and fastest route to this goal.

Gene function. Advances in molecular biology are most effectively driven by functional studies. The Human Genome Project should partition its resources between gene discovery and studies of the functions of the genes that are being discovered.

Genetic variation. Much of the biological interest in the human genome lies in generic variation and its relation to phenotype.

Model organisms. Basic cellular mechanisms can be studied more effectively in model organisms than in the human. The lessons learned in these systems are often readily transferable to the human because of the evolutionary conservation of critical genes. The list of model organisms under analysis could be expanded at modest cost since most model organisms have relatively small genomes.

Human resources. The development of genome centers and other laboratories with expertise in state-of-the-art methods is as important a goal as data collection. These laboratories are essential training resources and ensure widespread access to genome analysis tools. Continuity in the support of current programs should not be endangered by rapid shifts in programmatic emphasis.

These arguments underscore the need to maintain some balance amongst the Human Genome Project's diverse goals. They also make clear that genome analysis will face expanding, rather than contracting, opportunities once the human genome has been sequenced. Nonetheless, at the present juncture, the more compelling scientific and policy arguments favor a tightly focused Human Genome Project.

Genetic first principles favor early acquisition of a complete genomic sequence. The digital information that underlies biochemistry, cell biology, and development can be represented by a simple string of G's, A's, T's, and C's. This string is the root data structure of an organism's biology. Genetic and cytogenetic maps, as well as vast amounts of biochemical data, can be overlaid on the genome sequence in a natural way.

The financial costs of delay would exceed plausible savings from gains in efficiency. The Human Genome Project presently has a budget of approximately $200 million per year in the United States alone. The current cost of converting good STS maps to genomic sequence appears to be in the range of $0.20 to $0.40 per base pair. Costs will undoubtedly decline as economies of scale are realized. Hence, the total cost of producing a high-quality human sequence is likely to be less than $1 billion. Given present budgetary levels, the wait-and-see costs of an overly cautious policy would mount to $1 billion in just a few years. In all likelihood, the hidden costs of delayed availability of the data would be still larger because the sequence of the human genome would have broad effects on the efficiency of biomedical research.

Goal-oriented science projects are bad policy unless they have a well-defined objective. A vaguely defined Human Genome Program would be a bad compromise between targeted and investigator-initiated research. The more discipline that the project displays in setting priorities, the less it will threaten the curiosity-driven, small-laboratory science that is the best route to sustained scientific innovation. By shortening the path from observation to hypothesis to ex-

perimental test, the sequence of the human genome will empower small laboratories to attack problems in human biology that are presently beyond the reach of even the largest research teams.

International participation will be favored by an unequivocal commitment to very large-scale sequencing of human DNA. Different countries have diverse methods of organizing and supporting science. Efforts to negotiate common programs will collide with this diversity unless the goal and time schedule for a project are both clear. If the Human Genome Project in the United States moves decisively toward genomic sequencing, many other countries may be expected to join the effort, each mobilizing the needed support in its own way. The European yeast sequencing effort, spearheaded by the European Economic Community, achieved precisely this result after its pioneering commitment to obtain a complete sequence of the *Saccharomyces* genome. Increased international participation will allow sharing of the high financial cost of the Human Genome Project, while also securing a legacy of joint human participation in this important step in our genetic self-characterization.

Dynamic resource allocation works. The Human Genome Project in the United States has achieved consistent scientific success by allocating nearly all its resources through peer-reviewed grants that extend for 3 or 4 years. Competition within this system is intense, and many grants are not renewed even when they have met or exceeded their goals. This paradox is unavoidable in an applied science project with sequentially dependent objectives. While there are inefficiencies associated with this system, they pale beside those that result when permanent institutions are created that tie science to the past rather than the future.

There is a less abstract argument for moving ahead with human sequencing: That is what the money is for. The Human Genome Project was not sold to the U.S. Congress as a generalized vehicle for increasing support for molecular genetics, medical genetics, bioinformatics, or instrumentation development. It was sold on the grounds that sequencing the human genome would be immensely useful, was becoming technically feasible, and would not happen by itself. The foundations of this argument are worth revisiting. Substantial public resources have been invested in studies of the molecular genetics of model organisms. This investment was largely motivated by the perceived relevance of the research to human health. The Human Genome Project was designed both to make the human system easier to study directly and to increase the "bandwidth" for knowledge transfer between model-organism and human biology. The power of genome analysis to facilitate these goals is already well demonstrated (*14*). Completion of the sequence of the human genome and the sequences of the genomes of key model organisms will mobilize the full benefits of this new approach to biology.

While huge, the central task of the Human Genome Project is bounded by one of the most remarkable facts in all of science: The development of a human being is guided by just 750 megabytes of digital information. In vivo, this information is stored as DNA molecules in an egg or sperm cell. In a biologist's personal computer, it could be stored on a single CD-ROM. The Human Genome Project should get on with producing this disk, on time and under budget.

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The Minimal Gene Complement of *Mycoplasma genitalium*

Claire M. Fraser,* Jeannine D. Gocayne, Owen White, Mark D. Adams, Rebecca A. Clayton, Robert D. Fleischmann, Carol J. Bult, Anthony R. Kerlavage, Granger Sutton, Jenny M. Kelley, Janice L. Fritchman, Janice F. Weidman, Keith V. Small, Mina Sandusky, Joyce Fuhrmann, David Nguyen, Teresa R. Utterback, Deborah M. Saudek, Cheryl A. Phillips, Joseph M. Merrick, Jean-Francois Tomb, Brian A. Dougherty, Kenneth F. Bott, Ping-Chuan Hu, Thomas S. Lucier, Scott N. Peterson, Hamilton O. Smith, Clyde A. Hutchison III, J. Craig Venter

The complete nucleotide sequence (580,070 base pairs) of the *Mycoplasma genitalium* genome, the smallest known genome of any free-living organism, has been determined by whole-genome random sequencing and assembly. A total of only 470 predicted coding regions were identified that include genes required for DNA replication, transcription and translation, DNA repair, cellular transport, and energy metabolism. Comparison of this genome to that of *Haemophilus influenzae* suggests that differences in genome content are reflected as profound differences in physiology and metabolic capacity between these two organisms.

Mycoplasmas are members of the class Mollicutes, a large group of bacteria that lack a cell wall and have a characteristically low G + C content (*1*). These diverse organisms are parasites in a wide range of hosts including humans, animals, insects, plants, and cells grown in tissue culture (*1*). Aside from their role as potential pathogens, *Mycoplasmas* are of interest because of their reduced genome size and content relative to other prokaryotes.

Mycoplasma genitalium is thought to contain the smallest genome for a self-replicating organism (580 kb) and represents an important system for exploring a minimal functional gene set (*2*). *Mycoplasma genitalium* was originally isolated from urethral specimens of patients with non-gonoccocal urethritis (*3*) and has since been shown to exist in parasitic association with ciliated epithelial cells of primate genital and respiratory tracts (*4*).

The strategy and methodology for whole-genome random ("shotgun") sequencing and assembly was similar to that previously described for *Haemophilus influenzae* (*5*, *6*). To facilitate ordering of contigs, each template was sequenced from both ends. A total of 9846 sequencing reactions were performed by five individuals using an average of eight AB 373 DNA sequencers per day for a total of 8 weeks. Assembly of 8472 high-quality *M. genitalium* sequence fragments along with 299 random genomic sequences from Peterson *et al.* (*7*) was performed with the TIGR ASSEMBLER (*8*). The assembly process generated 39 contigs [size range, 606 to 73,351 base pairs (bp)] that contained a total of 3,806,280 bp of primary DNA sequence data. Contigs were ordered by ASM_ALIGN, a program that links contigs on the basis of information derived from forward and reverse sequencing reactions from the same clone.

ASM_ALIGN analysis revealed that all 39 gaps were spanned by an existing template from the small-insert genomic DNA library (that is, there were no physical gaps in the sequence assembly). The order of the contigs was confirmed by comparing the order of the random genomic sequences from Peterson *et al.* (*7*) that were incorporated into the assembly with their known position on the physical map of the *M. genitalium* chromosome (*9*). Because of the high stringency of the TIGR ASSEMBLER, the 39 contigs were searched against each other with GRASTA [a modified FASTA (*10*)] to detect overlaps (<30 bp) that would have been missed during the initial assembly process. Eleven overlaps were detected with this approach, which reduced the total number of gaps from 39 to 28.

Templates spanning each of the sequence gaps were identified, and oligonucleotide primers were designed from the sequences at the end of each contig. All gaps were less than 300 bp; thus, a primer walk from both ends of each template was sufficient for closure. All electropherograms were visually inspected with TIGR EDITOR (*5*) for initial sequence editing. Where a discrepancy could not be resolved or a clear assignment made, the automatic base calls were left unchanged. For each of the 53 ambiguities remaining after editing and the 25 potential frameshifts found after sequence-similarity searching, the appropriate template was resequenced with an alternative sequencing chemistry (dye terminator versus dye primer) to resolve ambiguities.

Ninety-nine percent of the *M. genitalium* genome was sequenced with better than single-sequence coverage, and the mean sequence redundancy was 6.5-fold. Although it is extremely difficult to assess sequence accuracy, we estimate our error rate to be less than 1 base in 10,000 on the basis of frequency of shifts in open reading frames (ORFs), overall quality of raw data, and fold coverage. The *M. genitalium* sequence (version 1.0) has been deposited in the Genome Sequence DataBase (GSDB) with the accession number L43967 (*11*).

Genome Analysis

The *M. genitalium* genome is a circular chromosome of 580,070 bp. The overall G + C content is 32% (A, 34%; C, 16%; G, 16%; and T, 34%). The G + C content across the genome varies between 27 and 37% (using a window of 5000 bp), with the regions of lowest G + C content flanking the presumed origin of replication for this organism (see below). As in *H. influenzae* (*5*), the ribosomal RNA (rRNA) operon (44%) and the transfer RNA (tRNA) genes (52%) in *M. genitalium* contain a higher G + C content than the rest of the genome, which may reflect the necessity of retaining essential G + C base pairing for secondary structure in rRNAs and tRNAs (*12*).

The genome of *M. genitalium* contains 74 Eco RI fragments, as predicted by both cosmid mapping data (*9*) and sequence analysis. The order and sizes of the Eco RI fragments determined by both methods are in agreement, with one apparent discrepancy between coordinates 62,708 and 94,573 in the sequence. However, reevaluation of

C. M. Fraser, J. D. Gocayne, O. White, M. D. Adams, R. A. Clayton, R. D. Fleischmann, C. J. Bult, A. R. Kerlavage, G. Sutton, J. M. Kelley, J. L. Fritchman, J. F. Weidman, K. V. Small, M. Sandusky, J. Fuhrmann, D. Nguyen, T. R. Utterback, D. M. Saudek, C. A. Phillips, and J. C. Venter are at the Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850, USA. J. M. Merrick is in the Department of Microbiology, State University of New York at Buffalo, Buffalo, NY 14214, USA. J.-F. Tomb, B. A. Dougherty and H. O. Smith are at the Johns Hopkins University School of Medicine, Baltimore, MD 21205, USA. K. F. Bott, P.-C. Hu, T. S. Lucier, S. N. Peterson, and C. A. Hutchison III are at the University of North Carolina at Chapel Hill, School of Medicine, Chapel Hill, NC 27599, USA.

*To whom correspondence should be addressed.

cosmid hybridization data in light of results from genome sequence analysis confirms that the sequence data are correct, and the extra 4.0-kb Eco RI fragment in this region of the cosmid map reflects a misinterpretation of the overlap between cosmids J-8 and 21 (*13*).

Studies of origins of replication in some prokaryotes have shown that DNA synthesis is initiated in an untranscribed AT-rich region between *dnaA* and *dnaN* (*14*). A search of the M. *genitalium* sequence for "DnaA boxes" around the putative origin of replication with consensus "DnaA boxes" from *Escherichia coli*, *Bacillus subtilis*, and *Pseudomonas aeruginosa* revealed no significant matches. Although we have not been able to localize the origin precisely, the colocalization of *dnaA* and *dnaN* to a 4000-bp region of the chromosome lends support to the hypothesis that it is the functional origin of replication in M. *genitalium* (*14–16*). The first base pair of the chromosomal sequence of M. *genitalium* is in an untranscribed region between *dnaA* and *dnaN* and was chosen so that *dnaN* is numbered as the first ORF in the genome. Genes to the right of this region are preferentially transcribed from the plus strand, and those to the left are preferentially transcribed from the minus strand. The apparent polarity in gene transcription is maintained across each half of the genome (Fig. 1), in marked contrast to *H. influenzae*, which displays no apparent polarity of transcription around the origin of replication.

The predicted coding regions of M. *genitalium* were initially defined by searching the entire genome for ORFs greater than 100 amino acids in length. Translations were made with the genetic code for mycoplasma species in which UGA encodes tryptophan. All ORFs were searched with BLAZE (*10*) against a nonredundant bacterial protein database (NRBP) (*5*) developed at TIGR on a MasPar MP-2 massively parallel computer with 4096 microprocessors. Protein matches were aligned with PRAZE, a modified Smith-Waterman (*17*) algorithm. Segments between predicted coding regions of the genome were also searched against all protein sequences from GenPept, Swiss-Prot, and the Protein Information Resource (PIR). The coding potential of 170 unidentified ORFs was analyzed with GeneMark (*18*), which had been trained with 308 M. *genitalium* sequences. Open reading frames that had low coding potential (on the basis of the GeneMark analysis) and were smaller than 100 nucleotides (a total of 53) were removed from the final set of putative coding regions. In a separate analysis, ORFs were searched against the complete set of translated sequences from *H. influenzae* [GSDB accession number L42023 (*5*)]. In total, these processes resulted in the identification of 470 predicted coding regions, of which 374 were putatively identified and 96 had no matches to protein sequences from any other organism. The 374 predicted coding regions with putative identifications were assigned biological roles with the classification system adapted from Riley (*19*).

Twenty-three of the protein matches in Table 1 have been annotated as motifs and represent matches where sequence similarity was confined to short domains in the predicted coding region. Several ORFs in M. *genitalium* displayed lower amino acid similarity to protein sequences in public

Fig. 1. Gene map of the *M. genitalium* genome. Predicted coding regions are shown, and the direction of transcription is indicated by arrows. Each line in the figure represents 24,000 bp of sequence in the *M. genitalium* genome. Genes are color-coded by role category as described in the key. Gene identificaton numbers correspond to those in Table 1. The rRNA operon, tRNA genes, and adhesin protein (MgPa) operon repeats are labeled.

archives than those observed with the motifs. In these cases, where motif identifications could not be made with confidence, the ORFs were annotated as no database match.

A separate search procedure was used in cases where we were unable to detect genes in the *M. genitalium* genome. Query peptide sequences that were available from eubacteria such as *E. coli*, *B. subtilis*, *Mycoplasma capricolum*, and *H. influenzae* were used in searches against all six reading frame translations of the entire genome sequence, and the alignments were examined by an experienced scientist. The possibility remains that current searching methods, an incomplete set of query sequences, or the subjective analysis of the database matches are not sensitive enough to identify certain *M. genitalium* gene sequences.

One-half of all predicted coding regions in *M. genitalium* for which a putative identification could be assigned display the greatest degree of similarity to a protein

Table 1. Summary of *M. genitalium* genes with putative identifications. Gene numbers correspond to those in Fig. 1. Each identified gene has been classified according to its role category [adapted from Riley (*19*)]. The putative gene identification and the percent amino acid identity are also listed for each entry. Those genes in *M. genitalium* that also match a gene in *H. influenzae* are indicated by an asterisk. An expanded version of this table with additional match information, including species, is available on the World Wide Web at URL http://www.tigr.org. Abbreviations: Bp, binding protein; DHase, dehydrogenase; G3PD, glyceraldehyde-3-phosphate dehydrogenase; MTase, methyltransferase; prt, protein; PRTase, phosphoribosyltransferase; Rdase, reductase; Tase, transferase; Sase, synthase; sub, subunit.

MG#	Identification	%ID
	Amino acid biosynthesis	
	Serine family	
*394	serine hydroxymethyltransferase (glyA)	55
	Biosynthesis of cofactors, prosthetic groups, and carriers	
	Folic acid	
*013	5,10-methylene-tetrahydrofolate DHase (folD)	33
*226	dihydrofolate RDase (dhfr)	33
	Heme and porphyrin	
*259	protoporphyrinogen oxidase (hemK)	31
	Thioredoxin, glutaredoxin, and glutathione	
*124	thioredoxin (trx)	36
*102	thioredoxin RDase (trxB)	39
	Cell envelope	
	Membranes, lipoproteins, and porins	
319	fibronectin-BP (fnbA)	25
040	membrane lipoprotein (tmpC)	31
*086	prolipoprotein diacylglyceryl Tase (lgt)	29
	Surface polysaccharides, lipopolysaccharides and antigens	
197	dTDP-4-dehydrorhemnose RDase (rfbD)	32
*358	lic-1 operon prt (licA) motif	28
*060	LPS biosyn prt (rfbV) motif	36
*269	surface prt antigen precursor (pag) motif	26
025	TrsB	28
	Surface structures	
192	114 kDa prt, MgPa operon (mgp)	100
191	attachment prt, MgPa operon (mgp)	100
315	cytadherence accessory prt (hmw1)	42
312	cytadherence-accessory prt (hmw1)	39
*386	cytadherence-accessory prt (hmw1)	34
313	cytadherence-accessory prt (hmw1)	53
*317	cytadherence-accessory prt (hmw3)	41
*459	surface exclusion prt (prgA) (Plasmid pCF10)	28
	Cellular processes	
	Cell division	
*457	cell division prt (ftsH)	50
*297	cell division prt (ftsY)	36
*224	cell division prt (ftsZ)	31
*434	mukB suppressor prt (smbA)	41
	Cell killing	
*146	hemolysin (tlyC)	26
220	pre-procytotoxin (vacA)	36
	Chaperones	
*019	heat shock prt (dnaJ)	34
002	heat shock prt (dnaJ) motif	40
*200	heat shock prt (dnaJ) motif	34
*392	heat shock prt (groEL)	52
*291	heat shock prt (grpE)	32
*393	heat shock prt 60-like prt (PggroES)	40
*305	heat shock prt 70 (hsp70)	57
	Detoxification	
*008	thiophene and furan oxidizer (tdhF)	32
	Protein and peptide secretion	
*138	GTP-binding membrane prt (lepA)	48
*179	haemolysin secretion ATP-BP (hlyB) motif	35
*072	preprotein translocase (secA)	44
*170	preprotein translocase secY sub (secY)	39
210	prolipoprotein signal peptidase (lsp)	32
*048	signal recognition particle prt (ffh)	43
	Transformation	
316	competence locus E (comE3) motif	30
	Central intermediary metabolism	
	Degradation of polysaccharides	
*217	bifunctional endo-1,4-beta-xylanase xyla precursor (xynA) motif	38
	Other	
*357	acetate kinase (ackA)	43
*038	glycerol kinase (glpK)	47
293	glycerophosphoryl diester phosphodiesterase (glpQ)	30
*299	phosphotransacetylase (pta)	45
	Phosphorus compounds	
*351	inorganic pyrophosphatase (ppa)	39
	Energy metabolism	
	Aerobic	
*039	glycerol-3-phospate DHase (GUT2)	43
*460	L-lactate DHase (ldh)	50
275	NADH oxidase (nox)	39
	ATP-proton motive force interconversion	
*405	adenosinetriphosphatase (atpB)	36
*401	ATP Sase alpha chain (atpA)	63
*403	ATP Sase B chain (atpF)	37
*399	ATP Sase beta chain (atpD)	61
*404	ATP Sase C chain (atpE)	50
*402	ATP Sase delta chain (atpH)	34
*398	ATP Sase epsilon chain (atpC)	37
*400	ATP Sase gamma chain (atpG)	38
	Glycolysis	
*063	1-phosphofructokinase (fruK)	26
*215	6-phosphofructokinase (pfk)	39
*407	enolase (eno)	54
*023	fructose-bisphosphate aldolase (tsr)	46
*301	G3PD (gap)	56
*111	phosphoglucose isomerase B (pgiB)	35
*300	phosphoglycerate kinase (pgk)	51
430	phosphoglycerate mutase (pgm)	45
*216	pyruvate kinase (pyk)	35
*431	triosephosphate isomerase (tim)	40
	Pentose phosphate pathway	
*264	6-phosphogluconate DHase (gnd)	30
*066	transketolase 1 (TK 1) (tktA)	33
	Pyruvate DHase	
*272	dihydrolipoamide acetyltransferase (pdhC)	45
*271	dihydrolipoamide DHase (pdhD)	38
*274	pyruvate DHase E1-alpha sub (pdhA)	43
*273	pyruvate DHase E1-beta sub (pdhB)	56
	Sugars	
*112	D-ribulose-5-phosphate 3 epimerase (cfxEc)	33
*050	deoxyribose-phosphate aldolase (deoC)	83
396	galactosidase acetyltransferase (lacA)	40
*053	phosphomannomutase (cpsG)	39
	Fatty acid and phospholipid metabolism	
*212	1-acyl-*sn*-glycerol-3-phosphate acetyltransferase (plsC)	32
*437	CDP-diglyceride Sase (cdsA)	38
368	fatty acid/phospholipid synthesis prt (plsX)	29
095	hydroxymethylglutaryl-CoA RDase (NADPH)	23
344	lipase-esterase (lip1)	27
*114	phosphatidylglycerophosphate Sase (pgsA)	29
	Purines, pyrimidines, nucleosides, and nucleotides	
	2'-Deoxyribonucleotide metabolism	
*231	ribonucleoside-diphosphate RDase (nrdE)	54
*229	ribonucleoside RDase 2 (nrdF)	50
*227	thymidylate Sase (thyA)	57
	Nucleotide and nucleoside interconversions	
*382	uridine kinase (udk)	34
	Purine ribonucleotide biosynthesis	
*107	5'-guanylate kinase (gmk)	43
*171	adenylate kinase (adk)	32
*058	phosphoribosylpyrophosphate Sase (prs)	44
	Salvage of nucleosides and nucleotides	
*276	adenine PRTase (apt)	34
*052	cytidine deaminase (cdd)	38
*330	cytidylate kinase (cmk)	40
268	deoxyguanosine-deoxyadenosine kinase(i) sub 2	30
*458	hypoxanthine-guanine PRTase (hpt)	38
*049	purine-nucleoside phosphorylase (deoD)	44
*034	thymidine kinase (tdk)	48
051	thymidine phosphorylase (deoA)	53
*006	thymidylate kinase (CDC8)	28
*030	uracil PRTase (upp)	45
	Sugar-nucleotide biosynthesis and conversions	
*118	UDP-glucose 4-epimerase (galE)	34
*453	UDP-glucose pyrophosphorylase (gtaB)	48
	Regulatory functions	
*024	GTP-BP (gtp1)	47
*384	GTP-BP (obg)	40
*387	GTP-BP (era)	27
248	major sigma factor (rpoD)	28
*448	pilin repressor (pilB)	53
*408	pilin repressor (pilB) motif	49
*104	virulence-associated prt homolog (vacB)	29
	Replication	
	Degradation of DNA	
032	ATP-dependent nuclease (addA)	27
	DNA replication, restriction, modification, recombination, and repair	
*469	chromosomal replication initiator prt (dnaA)	31
*004	DNA gyrase sub A (gyrA)	100
*003	DNA gyrase sub B (gyrB)	99
*244	DNA helicase II (mutB1)	36
*254	DNA ligase (lig)	36
*262	DNA polymerase I (polI) motif	30
*031	DNA polymerase III (polC)	38
*261	DNA polymerase III alpha sub (dnaE)	32
*001	DNA polymerase III beta sub (dnaN)	100
*420	DNA polymerase III sub (dnaH)	49
007	DNA polymerase III sub (dnaH) motif	23
*250	DNA primase (dnaE)	27
*010	DNA primase (dnaE) motif	26
*122	DNA topoisomerase I (topA)	39
235	endonuclease IV (nfo)	29
*421	excinuclease ABC sub A (uvrA)	48
*073	excinuclease ABC sub B (uvrB)	48
*206	excinuclease ABC sub C (uvrC)	28
*379	glucose-inhibited division prt (gidA)	40
*380	glucose-inhibited division prt (gidB)	25
*358	Holliday junction DNA helicase (ruvA)	26
*359	Holliday junction DNA helicase (ruvB)	35
184	MTase (ssoIM)	43
*339	recombination prt (recA)	47
*094	replicative DNA helicase (dnaB)	33
438	restriction-modification enzyme EcoD specificity sub (hsdS)	25
*047	S-adenosylmethionine Sase 2 (metX)	44
*091	single-stranded DNA BP (ssb)	22
*204	DNA topoisomerase IV sub A (parC)	100
*203	DNA topoisomerase IV sub B (parE)	100
*097	uracil DNA glycosylase (ung)	33
	Transcription	
	Degradation of RNA	
*367	ribonuclease III (rnc)	30
*465	RNase P C5 sub (rnpA)	40
	RNA synthesis, modification, and DNA transcription	
*308	ATP-dependent RNA helicase (deaD)	23
*425	ATP-dependent RNA helicase (deaD)	32
018	helicase (mot1) motif	44
*141	N-utilization substance prt A (nusA)	36
*177	RNA polymerase alpha core sub (rpoA)	31
*341	RNA polymerase beta sub (rpoB)	39
*340	RNA polymerase beta' chain (rpoC)	41
022	RNA polymerase delta sub (rpoE)	29
*249	RNA polymerase sigma A factor (sigA)	44
*054	transcription antitermination factor (nusG)	31
	Translation	
	Amino acyl tRNA synthetases and tRNA modification	
*292	Ala-tRNA Sase (alaS)	34
*376	Arg-tRNA Sase (argS)	34
*113	Asn-tRNA Sase (asnS)	41
*036	Asp-tRNA Sase (aspS)	41
*253	Cys-tRNA Sase (cysS)	34
*462	Glu-tRNA Sase (gltX)	43
251	Gly-tRNA Sase	36
*035	His-tRNA Sase (hisS)	31
*345	Ile-tRNA Sase (ileS)	33
*266	Leu-tRNA Sase (leuS)	43
*136	Lys-tRNA Sase (lysS)	46
*365	Met-tRNA formyltransferase (fmt)	36
*021	Met-tRNA Sase (metS)	36
*083	peptidyl-tRNA hydrolase homolog (pth)	38
*195	Phe-tRNA Sase alpha chain (pheT)	26
*194	Phe-tRNA Sase beta chain (pheS)	35
*283	Pro-tRNA Sase (proS)	23
*182	pseudouridylate Sase I (hisT)	37
*005	Ser-tRNA Sase (serS)	43
*375	Thr-tRNA Sase (thrSv)	39
*445	tRNA (guanine-N1)-MTase (trmD)	41
*126	Trp-tRNA Sase (trpS)	41
*455	Tyr-tRNA Sase (tyrS)	39
*334	Val-tRNA Sase (valS)	39
	Degradation of proteins, peptides, and glycopeptides	
*391	aminopeptidase	45
*324	aminopeptidase P (pepP)	31
*239	ATP-dependent protease (lon)	44
*355	ATP-dependent protease binding sub (clpB)	46
067	glutamic acid specific protease (SPase)	29
219	IgA1 protease	32
183	oligoendopeptidase F (pepF)	30
020	proline iminopeptidase (pip)	38
310	proline iminopeptidase (pip)	29
*048	sialoglycoprotease (gcp)	36
*238	trigger factor (tig)	26
	Protein modification and translation factors	
*089	elongation factor G (fus)	59
*026	elongation factor P (efp)	26
*433	elongation factor Ts (tsf)	39
*451	elongation factor TU (tuf)	100
*106	formylmethionine deformylase (def) motif	37
*173	initiation factor 1 (infA)	49
*172	methionine amino peptidase (map)	36
*258	peptide chain release factor 1 (RF-1)	43
108	prt phosphatase 2C homolog (ptc1) motif	28
109	prt serine-threonine kinase motif	34
*142	prt synthesis initiation factor 2 (infB)	46
*435	ribosome releasing factor (frr)	35
*282	transcription elongation factor (greA)	40
*196	translation initiation factor IF3 (infC)	31
	Ribosomal proteins: synthesis and modification	
*082	ribosomal prt L1	46
*361	ribosomal prt L10	30
*081	ribosomal prt L11	52
*418	ribosomal prt L13	40
*161	ribosomal prt L14	63
*169	ribosomal prt L15	42
*158	ribosomal prt L16	64
*178	ribosomal prt L17	35
*187	ribosomal prt L18	43
*444	ribosomal prt L19	49
*154	ribosomal prt L2	58
*198	ribosomal prt L20	58
*232	ribosomal prt L21	38
233	ribosomal prt L21 homolog	100
*156	ribosomal prt L22	49
153	ribosomal prt L23	45
*162	ribosomal prt L24	64
*234	ribosomal prt L27	36
*426	ribosomal prt L28	42
*159	ribosomal prt L29	43
*151	ribosomal prt L3	37
*257	ribosomal prt L31	48
*363	ribosomal prt L32	58
*325	ribosomal prt L33	46
*466	ribosomal prt L34	67
197	ribosomal prt L35	60
174	ribosomal prt L36	78
*152	ribosomal prt L4	39
*163	ribosomal prt L5	58
*166	ribosomal prt L6	46
*382	ribosomal prt L7/L12 ('A' type)	49
*093	ribosomal prt L9	33
*150	ribosomal prt S10	49
*176	ribosomal prt S11	48
*087	ribosomal prt S12	75
*175	ribosomal prt S13	63
*164	ribosomal prt S14	70
*424	ribosomal prt S15	48
*446	ribosomal prt S16	49
*160	ribosomal prt S17	51
*092	ribosomal prt S18	45
*155	ribosomal prt S19	59
*070	ribosomal prt S2	35
*157	ribosomal prt S3	47
*311	ribosomal prt S4	43
*168	ribosomal prt S5	56
*090	ribosomal prt S6	24
*012	ribosomal prt S6 modification prt (rimK) motif	31
*088	ribosomal prt S7	65
*165	ribosomal prt S8	47
*417	ribosomal prt S9	52
*252	rRNA methylase	39
	Transport and binding proteins	
	Amino acids, peptides, and amines	
226	aromatic amino acid transport prt (aroP)	25
*180	membrane transport prt (glnQ)	37
*303	membrane transport prt (glnQ)	32
*079	oligopeptide transport ATP-BP (amiE)	46
*080	oligopeptide transport ATP-BP (amiF)	47
*078	oligopeptide transport permease prt (dciAC)	33
*077	oligopeptide transport permease prt (oppB)	26
*042	spermidine-putrescine transport ATP-BP (potA)	42
*043	spermidine-putrescine transport permease prt (potB)	27
*044	spermidine-putrescine transport permease prt (potC)	29
	Anions	
*410	peripheral membrane prt B (pstB)	51
409	peripheral membrane prt U (phoU)	27
*411	periplasmic phosphate permease homolog (pstS8)	31
	Carbohydrates, organic alcohols, and acids	
*187	ATP-BP (msmK)	41
*062	fructose-permease IIBC component (fruA)	43
*033	glycerol uptake facilitator (glpF)	36
061	hexosephosphate transport prt (uhpT)	31
*168	membrane prt (msmF)	22
189	membrane prt (msmG)	27
*119	methylgalactoside permease ATP-BP (mglA)	33
*429	PEP-dependent HPr prt kinase phosphorylttransferase (ptsI)	46
*041	phosphohistidinoprotein-hexose phosphotransferase (ptsH)	49
*069	phosphotransferase enzyme II, ABC component (ptsG)	43
129	PTS glucose-specific permease	29
*120	ribose transport permease prt (rbsC)	27
	Cations	
071	cation-transporting ATPase (pacL)	34
	Other	
*290	ATP-BP P29	32
269	high affinity transport prt P37 (P37)	36
*390	lactococcin transport ATP-BP (lcnDR3)	22
322	Na^+ ATPase sub J (ntpJ)	31
*014	transport ATP-BP (msbA)	28
*015	transport ATP-BP (msbA)	32
291	transport permease prt P69 (P69)	28
*406	transport permease prt P69 (P69) motif	40
	Other categories	
	Adaptations and atypical conditions	
454	osmotically inducible prt (osmC)	28
	phosphate limitation prt (sphX)	31
470	SpoOJ regulator motif	27
*277	spore germination apparatus prt (gerBB) motif	31
383	sporulation prt (outB) motif	36
	Drug and analog sensitivity	
*463	high-level kasgamycin resistance (ksgA)	36
	Other	
*298	115 kD prt (p115)	33
190	29 kDa prt, MgPa operon (mgp)	62
*065	heterocyst maturation prt (devA)	35
*467	heterocyst maturation prt (devA)	40
099	hydrolase (aux2)	32
131	hypothetical prt (GB:M31161_3)	22
*218	macroglobin	25
*327	magnesium chelatase 30 kD sub (bchO)	27
*304	membrane-associated ATPase (cbiO)	30
364	mobilization prt (mob13) motif	31
*336	nitrogen fixation prt (nifS)	26
267	nodulation prt F (nodF)	35
098	p48 eggshell prt (p48)	23
100	PET112 prt	31
037	pre-B cell enhancing factor (PBEF)	34
268	prt L	28
*328	prt V (tcrV)	28
*145	prt X	29
*280	sensory rhodopsin II transducer (htrII) motif	16
*059	small prt (smpB)	33
360	UV protection prt (mucB)	22

from either a Gram-positive organism (for example, *B. subtilis*) or a *Mycoplasma* species. The significance of this finding is underscored by the fact that NRBP contains 3885 sequences from *E. coli* and only 1975 sequences from *B. subtilis*. In the majority of cases where *M. genitalium* coding regions matched sequences from both *E. coli* and *Bacillus* species, the better match was to a sequence from *Bacillus* (average, 62% similarity) rather than to a sequence from *E. coli* (average, 56% similarity). The evolutionary relationship between *Mycoplasma* and the *Lactobacillus-Clostridium* branch of the Gram-positive phylum has been deduced from small-subunit rRNA sequences (*20*, *21*). Our data from whole-genome analysis support this hypothesis.

Comparative Genomics: *M. genitalium* and *H. influenzae*

A survey of the genes and their organization in *M. genitalium* permits the description of a minimal set of genes required for survival. One would predict that a minimal cell must contain genes for replication and transcription, at least one rRNA operon and a set of ribosomal proteins, tRNAs and tRNA synthetases, transport proteins to derive nutrients from the environment, biochemical pathways to generate adenosine triphosphate (ATP) and reducing power, and mechanisms for maintaining cellular homeostasis. Comparison of the genes identified in *M. genitalium* with those in *H. influenzae* allows for identification of a basic complement of genes conserved in these two species and provides insights into physiological differences between one of the simplest self-replicating prokaryotes and a more complex, Gram-negative bacterium.

The *M. genitalium* genome contains 470 predicted coding sequences as compared with 1727 identified in *H. influenzae* (*5*) (Table 2). The percent of the total genome in *M. genitalium* and *H. influenzae* that encodes genes involved in cellular processes, central intermediary metabolism, energy metabolism, fatty acid and phospholipid metabolism, purine and pyrimdine metabolism, replication, transcription, transport, and other categories is similar, although the total number of genes in these categories is considerably fewer in *M. genitalium*. A smaller percentage of the *M. genitalium* genome encodes genes involved in amino acid biosynthesis, biosynthesis of cofactors, cell envelope, and regulatory functions as compared with *H. influenzae*. A greater percentage of the *M. genitalium* genome encodes proteins involved in translation than in *H. influenzae*, as shown by the similar numbers of ribosomal proteins and tRNA synthetases in both organisms.

The 470 predicted coding regions in *M. genitalium* (average size, 1040 bp) comprise 88% of the genome (on average, one gene every 1235 bp), a value similar to that found in *H. influenzae* where 1727 predicted coding regions (average size, 900 bp) comprise 85% of the genome (one gene every 1042 bp). These data indicate that the reduction in genome size that has occurred in *Mycoplasma* has not resulted in an increase in gene density or a decrease in gene size (*22*). A global search of *M. genitalium* and *H. influenzae* genomes reveals short regions of conservation of gene order, particularly two clusters of ribosomal proteins.

Replication. We have identified genes that encode many essential proteins in the replication process, including *M. genitalium* isologs of the proteins DnaA, DnaB, GyrA,

Table 2. Summary of gene content in *H. influenzae* and *M. genitalium* sorted by functional category. The number of genes in each functional category is listed for *H. influenzae* and *M. genitalium*. The number in parentheses indicates the percent of the putatively identified genes devoted to each functional category. For the category of unassigned genes, the percent of the genome indicated in parentheses represents the percent of the total number of putative coding regions.

Biological role	*H. influenzae*	*M. genitalium*
Amino acid biosynthesis	68 (6.8)	1 (0.3)
Biosynthesis of cofactors	54 (5.4)	5 (1.6)
Cell envelope	84 (8.3)	17 (5.3)
Celluar processes	53 (5.3)	21 (6.6)
Cell division	16	4
Cell killing	5	2
Chaperones	6	7
Detoxification	3	1
Protein secretion	15	6
Transformation	8	1
Central intermediary metabolism	30 (3)	6 (1.9)
Energy metabolism	112 (10.4)	31 (9.7)
Aerobic	4	3
Amino acids and amines	4	0
Anaerobic	24	0
ATP–proton force interconversion	9	8
Electron transport	9	0
Entner-Doudoroff	9	0
Fermentation	8	0
Gluconeogenesis	2	0
Glycolysis	10	10
Pentose phosphate pathway	3	2
Pyruvate dehydrogenase	4	4
Sugars	15	4
TCA cycle	11	0
Fatty acid and phospholipid metabolism	25 (2.5)	6 (1.9)
Purines, pyrimidines, nucleosides, and nucleotides	53 (5.3)	19 (6.0)
2′-Deoxyribonucleotide metabolism	8	3
Nucleotide and nucleoside interconversions	3	1
Purine ribonucleotide biosynthesis	18	3
Pyrimidine ribonucleotide biosynthesis	5	0
Salvage of nucleosides and nucleotides	13	10
Sugar-nucleotide biosynthesis and conversions	6	2
Regulatory functions	64 (6.3)	7 (2.2)
Replication	87 (8.6)	32 (10.0)
Degradation of DNA	8	1
DNA replication, restriction, modification, recombination, and repair	76	31
Transcription	27 (2.7)	12 (3.8)
Degradation of RNA	10	2
RNA synthesis and modification, DNA transcription	17	10
Translation	141 (14)	101 (31.8)
Transport and binding proteins	123 (12.2)	34 (10.7)
Amino acids and peptides	38	10
Anions	8	3
Carbohydrates	30	12
Cations	24	1
Other transporters	22	8
Other categories	93 (9.2)	27 (8.2)
Unassigned role	736 (43)	152 (32)
No database match	389	96
Match hypothetical proteins	347	56

GyrB, a single-stranded DNA-binding protein, and the primase protein DnaE. DnaJ and DnaK, heat shock proteins that may function in the release of the primosome complex, are also found in *M. genitalium*. A gene encoding the DnaC protein, responsible for delivery of DnaB to the primosome, has yet to be identified.

Genes encoding most of the essential subunit proteins for DNA polymerase III in *M. genitalium* were also identified. The *polC* gene encodes the α subunit, which contains the polymerase activity. We have also identified the isolog of *dnaH* in *B. subtilis* (dnaX in *E. coli*) that encodes the γ and τ subunits as alternative products from the same gene. These proteins are necessary for the processivity of DNA polymerase III. An isolog of *dnaN* that encodes the β subunit was previously identified in *M. genitalium* (*15*) and is involved in the process of clamping the polymerase to the DNA template. While we have yet to identify a gene encoding the ε subunit responsible for the 3′-5′ proofreading activity, it is possible that this activity is encoded in the α subunit as previously described (*23*). Finally, we have identified a gene encoding a DNA ligase, necessary for the joining of the Okazaki fragments formed during synthesis of the lagging strand.

While we have identified genes encoding many isologs thought to be essential for DNA replication, some genes encoding proteins with key functions have yet to be identified. Examples of these are Dnaθ and Dnaδ, whose functions are less well understood but are thought to be involved in the assembly and processivity of polymerase III. Also apparently absent is a specific ribonuclease H protein responsible for the hydrolysis of the RNA primer synthesized during lagging-strand synthesis.

DNA repair. It has been suggested that in *E. coli* as many as 100 genes are involved in DNA repair (*24*), and in *H. influenzae* the number of putatively identified DNA-repair enzymes is approximately 30 (*5*). Although *M. genitalium* appears to have the necessary genes to repair many of the more common lesions in DNA, the number of genes devoted to the task is much smaller. Excision repair of regions containing missing bases [apurinic or apyriminic (AP) sites] can likely occur by a pathway involving endonuclease IV (nfo), Pol I, and ligase. The *ung* gene, which encodes uracil-DNA glycosylase, is present. This activity removes uracil residues from DNA that usually arise by spontaneous deamination of cytosine.

All three genes necessary for production of the ultraviolet-resistant ABC exinuclease are present, and along with Pol I, helicase II, and ligase should provide a mechanism for repair of damage such as cross-linking, which requires replacement of both strands. Although *recA* is present, which in *E. coli* is activated as it binds to single-stranded DNA, thereby initiating the SOS response, we find no evidence for a *lexA* gene, which encodes the repressor that regulates the SOS genes. We have not identified photolyase (*phr*) in *M. genitalium*, which repairs ultraviolet-induced pyrimidine dimers, or other genes involved in reversal of DNA damage rather than excision and replacement of the lesion.

Transcription. The critical components for transcription were identified in *M. genitalium*. In addition to the α, β, and β′ subunits of the core RNA polymerase, *M. genitalium* appears to encode a single σ factor, whereas *E. coli* and *B. subtilis* encode at least six and seven, respectively. We have not detected a homolog of the Rho termination factor gene, so it seems likely that a mechanism similar to Rho-independent termination in *E. coli* operates in *M. genitalium*. We have clear evidence for homologs of only two other genes that modulate transcription, *nusA* and *nusG*.

Translation. *Mycoplasma genitalium* has a single rRNA operon that contains three rRNA subunits in the order 16*S* rRNA (1518 bp)–spacer (203 bp)–23*S* rRNA (2905 bp)–spacer (56 bp)–5*S* rRNA (103 bp). The small-subunit rRNA sequence was compared with the Ribosomal Database Project's (*21*) prokaryote database with the program "similarity_rank." Our sequence is identical to the *M. genitalium* (strain G37) sequence deposited there, and the 10 most similar taxa returned by this search are also in the genus *Mycoplasma*.

A total of 33 tRNA genes were identified in *M. genitalium*; these were organized into five clusters plus nine single genes. In all cases, the best match for each tRNA gene in *M. genitalium* was the corresponding gene in *M. pneumoniae* (*25*). Furthermore, the grouping of tRNAs into clusters (trnA, trnB, trnC, trnD, and trnE) was identical in *M. genitalium* and *M. pneumoniae*, as was gene order within the cluster (*25*). The only difference between *M. genitalium* and *M. pneumoniae* with regard to tRNA gene organization was an inversion between trnD and GTG. In contrast to *H. influenzae* and many other eubacteria, no tRNAs were found in the spacer region between the 16*S* and 23*S* rRNA genes in the rRNA operon of *M. genitalium*, similar to what has been reported for *M. capricolum* (*26*).

A search of the *M. genitalium* genome for tRNA synthetase genes identified all of the expected genes except glutaminyl tRNA synthetase (*glnS*). In *B. subtilis* and other Gram-positive bacteria, and *Saccharomyces cerevisiae* mitochondria, no glutaminyl tRNA synthetase activity has been detected (*27*). In these organisms, a single glutamyl tRNA synthetase aminoacylates both $tRNA^{Glu}$ and $tRNA^{Gln}$ with glutamate (*28*). The formation of glutaminyl tRNA synthetase is accomplished by amidation of glutamate to glutamine in a reaction that is functionally analogous to that catalyzed by glutamine synthetase (*29*). Because of its evolutionary relationship with Gram-positive organisms (*20*, *21*), it is likely that a similar mechanism is involved in the formation of glutaminyl tRNA synthetase in *M. genitalium*.

Metabolic pathways. The reduction in genome size among *Mycoplasma* species is associated with a marked reduction in the number and components of biosynthetic pathways in these organisms, thereby requiring them to use metabolic products from their hosts. The complex growth requirements of this organism in the laboratory can be explained by the almost complete lack of enzymes involved in amino acid biosynthesis, de novo nucleotide biosynthesis, and fatty acid biosynthesis (Table 1 and Fig. 1). When the number of genes in the categories of central intermediary metabolism, energy metabolism, and fatty acid and phospholipid metabolism are examined, marked differences in gene content between *H. influenzae* and *M. genitalium* are apparent. For example, whereas the *H. influenzae* genome contains 68 genes involved in amino acid biosynthesis, the *M. genitalium* genome contains only 1. In total, the *H. influenzae* genome has 228 genes associated with metabolic pathways, whereas the *M. genitalium* genome has just 44. A recent analysis of 214 kb of sequence from *Mycoplasma capricolum* (*22*), a related organism whose genome size is twice as large as that of *M. genitalium*, reveals that *M. capricolum* contains a number of biosynthetic enzymes not present in *M. genitalium*. This observation suggests that *M. capricolum*'s larger genome confers a greater anabolic capacity.

Mycoplasma genitalium is a facultative anaerobe that ferments glucose and possibly other sugars by way of glycolysis to lactate and acetate. Genes that encode all the enzymes of the glycolytic pathway were identified, including genes for components of the pyruvate dehydrogenase complex, phosphotransacetylase, and acetate kinase. The major route for ATP synthesis may be through substrate-level phosphorylation, because no cytochromes are present. *Mycoplasma genitalium* also lacks all the components of the tricarboxylic acid cycle. None of the genes encoding glycogen or poly-β-hydroxybutryate production were identified, indicating limited capacity for carbon and energy storage. The pentose phosphate pathway also appears limited because only genes encoding 6-phosphogluconate dehydrogenase and transketolase were identified. The limited metabolic capacity of *M. genitalium* contrasts sharply with the complexity of cata-

bolic pathways in *H. influenzae*, reflecting the fourfold greater number of genes involved in energy metabolism found in *H. influenzae*.

Transport. The transporters identified in *M. genitalium* are specific for a range of nutritional substrates. In protein transport, for example, both oligopeptide and amino acid transporters are represented. One interesting peptide transporter is similar to a lactococcin transporter (lcnDR3) and related bacteriocin transporters, suggesting that *M. genitalium* may export a small peptide with antibacterial activity. The *M. genitalium* isolog of the *M. hyorhinis* p37 high-affinity transport system also has a conserved lipid-modification site, providing further evidence that the *Mycoplasma* binding protein–dependent transport systems are organized in a manner analogous to Gram-positive bacteria (*30*).

Genes encoding proteins that function in the transport of glucose by way of the phosphoenolpyruvate:sugar transferase system (PTS) have been identified in *M. genitalium*. These proteins include enzyme I (EI), HPr, and sugar-specific enzyme IIs (EIIs) (*31*). EIIs is a complex of at least three domains: EIIA, EIIB, and EIIC. In some bacteria (for example, *E. coli*) EIIA is a soluble protein, whereas in others (*B. subtilis*) a single membrane protein contains all three domains. These variations in the proteins that make up the EII complex are due to fusion or splitting of domains during evolution and are not considered to be mechanistic differences (*31*). In *M. genitalium*, EIIA, -B, and -C are located in a single protein similar to that found in *B. subtilis*. In *M. capricolum ptsH*, the gene that encodes HPr is located on a monocistronic transcriptional unit, whereas genes encoding EI (*ptsI*) and EIIA (*crr*) are located on a dicistronic operon (*32*). In most bacterial species studied to date, *ptsI*, *ptsH*, and *crr* are part of a polycistonic operon (*pts* operon). In *M. genitalium*, *ptsH*, *ptsI*, and the gene encoding EIIABC reside at different locations of the genome, and thus each of these genes may constitute monocistronic transcriptional units. We have also identified the EIIBC component for uptake of fructose; however, other components of the fructose PTS were not found. Thus, *M. genitalium* may be limited to the use of glucose as an energy source. In contrast, *H. influenzae* has the ability to use at least six different sugars as a source of carbon and energy.

Regulatory systems. It appears that regulatory systems found in other bacteria are absent in *M. genitalium*. For instance, although two-component systems have been described for a number of Gram-positive organisms, no sensor or response regulator genes are found in the *M. genitalium* genome. Furthermore, the lack of a heat shock σ factor raises the question of how the heat shock response is regulated. Another stress faced by all metabolically active organisms is the generation of reactive oxygen intermediates such as superoxide anions and hydrogen peroxide. Although *H. influenzae* has an *oxyR* homolog, as well as catalase and superoxide dismutase, *M. genitalium* appears to lack these genes as well as an NADH [nicotinamide adenine dinucleotide (reduced)] peroxidase. The importance of these reactive intermediate molecules in host cell damage suggests that some as yet unidentified protective mechanism may exist within the cell.

Antigenic variation. The 140-kD adhesin protein of *M. genitalium* is densely clustered at a differentiated tip and elicits a strong immune response in humans and experimentally infected animals (*33*). The adhesin protein (MgPa) operon in *M. genitalium* contains a 29-kD ORF, the MgPa protein (160 kD), and a 114-kD ORF with intervening regions of six nucleotides and one nucleotide, respectively (*34*). On the basis of hybridization experiments (*35*), multiple copies of regions of the *M. genitalium* MgPa gene and the 114-kD ORF are known to exist throughout the genome.

The availability of the complete genomic sequence from *M. genitalium* has allowed a comprehensive analysis of the MgPa repeats. In addition to the complete operon, nine repetitive elements that are composites of regions of the MgPa operon were found. (Fig. 1) The percent of sequence identity between the repeat elements and the MgPa operon genes ranges from 78 to 90%. The sequences contained in the MgPa operon and the nine repeats scattered throughout the chromosome represent 4.7% of the total genomic sequence. Although this observation might appear to contradict the expectation for a minimal genome, recent evidence for recombination between the repetitive elements and the MgPa operon has been reported (*36*). Such recombination may allow *M. genitalium* to evade the host immune response through mechanisms that induce antigenic variation within the population.

The *M. genitalium* genome contains 90 putatively identified genes that do not appear to be present in *H. influenzae*. Almost 60% of these genes have database matches to known or hypothetical proteins from Gram-positive bacteria or other *Mycoplasma* species, suggesting that these genes may encode proteins with a restricted phylogenetic distribution. Ninety-six potential coding regions in *M. genitalium* have no database match to any sequences in public archives including the entire *H. influenzae* genome; therefore, these likely represent novel genes in *M. genitalium* and related organisms.

The predicted coding sequences of the hypothetical ORFs, the ORFs with motif matches, and the ORFs that have no similarities to known peptide sequences were analyzed with the Kyte-Doolittle algorithm (*37*), with a range of 11 residues, and PSORT, which is available on the World Wide Web at URL http://psort.nibb.ac.jp. PSORT predicts the presence of signal sequences by the methods of McGeoch and von Heijne (*38*), and detects potential transmembrane domains by the method of Klein *et al.* (*39*). Of a total of 175 ORFs examined, 90 potential membrane proteins were found, 11 of which were predicted to have type I signal peptides and 5 to have type II signal peptides. At least 50 potential membrane proteins with role assignments were also identified by this approach, in agreement with previously predicted or confirmed membrane localizations for these proteins. Taken together, these data suggest that the total number of potential membrane proteins in *M. genitalium* may be on the order of 140.

To manage these putative membrane proteins, *M. genitalium* has at its disposal a minimal secretory machinery composed of six functions: two chaperonins (GroEL and DnaK) (*40*, *41*), an adenosine triphosphatase (ATPase) pilot protein (SecA), one integral membrane protein translocase (SecY), a signal recognition particle protein (Ffh), and a lipoprotein-specific signal peptidase (LspA) (*40*). Perhaps the lack of other known translocases (like SecE, SecD, and SecF) that are present in *E. coli* and *H. influenzae* is related to the presence in *M. genitalium* of a one-layer cell envelope. Also, the absence in *M. genitalium* of a SecB homolog, the secretory chaperonin of *E. coli* [it is also absent in *B. subtilis* (*42*)], might reflect a difference between Gram-negative and wall-less Mollicutes in processing nascent proteins destined for the general secretory pathway. Considering the presence of several putative membrane proteins that contain type I signal peptides, the absence of a signal peptidase I (*lepB*) is most surprising. A direct electronic search for the *M. genitalium lepB* gene with the *E. coli lepB* and the *B. subtilis sipS* (*43*) as queries did not reveal any signficant similarities.

A number of possibilities may explain why genes encoding some of the proteins characterized in other eubacterial species appear to be absent in *M. genitalium*. One possibility is that a limited number of proteins in this organism may have become adapted to perform other functions. A second possibility is that certain proteins found in more complex bacteria such as *E. coli* are not required in a simpler prokaryote like *M. genitalium*. Finally, sequences from *M. genitalium* may have such low similarity to known sequences from other species that matches are not detectable above a reason-

able confidence threshold.

The complete sequencing and assembly of other microbial genomes, together with genome surveys using random sequencing, will continue to provide a wealth of information on the evolution of single genes, gene families, and whole genomes. Comparison of these data with the genome sequence of *M. genitalium* should allow a more precise definition of the fundamental gene complement for a self-replicating organism and a more comprehensive understanding of the diversity of life.

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Transfer of Genes to Humans: Early Lessons and Obstacles to Success

Ronald G. Crystal

Enough information has been gained from clinical trials to allow the conclusion that human gene transfer is feasible, can evoke biologic responses that are relevant to human disease, and can provide important insights into human biology. Adverse events have been uncommon and have been related to the gene delivery strategies, not to the genetic material being transferred. Human gene transfer still faces significant hurdles before it becomes an established therapeutic strategy. However, its accomplishments to date are impressive, and the logic of the potential usefulness of this clinical paradigm continues to be compelling.

Human gene transfer is a clinical strategy in which the genetic repertoire of somatic cells is modified for therapeutic purposes or to help gain understanding of human biology (*1*, *2*). Essentially, gene transfer involves the delivery, to target cells, of an expression cassette made up of one or more genes and the sequences controlling their expression. This can be carried out ex vivo in a procedure in which the cassette is transferred to cells in the laboratory and the modified cells are then administered to the recipient. Alternatively, human gene transfer can be done in vivo, in a procedure in which the expression cassette is transferred directly to cells within an individual. In both strategies, the transfer process is usually aided by a vector that helps deliver the cassette to the intracellular site where it can function appropriately (*1*, *2*).

Once considered a fantasy that would not become reality for generations, human gene transfer moved from feasibility and safety studies in animals to clinical applications more rapidly than expected by even its most ardent supporters (*1*–*3*). It is not the purpose of this review to detail all human protocols that have been proposed, but to use examples from the available information regarding ongoing human trials (*3*) to define the current status of the field.

How Is Human Gene Transfer Carried Out?

The choice of an ex vivo or in vivo strategy and of the vector used to carry the expression cassette is dictated by the clinical target. The vector systems for which data are available from clinical trials (retroviruses, adenoviruses, and plasmid-liposome complexes) transfer expression cassettes through different mechanisms and thus have distinct advantages and disadvantages for different applications (*1*, *2*).

Vectors. Replication-deficient, recombinant retrovirus vectors can accommodate up to 9 kb of exogenous information (Fig. 1). Retroviruses transfer their genetic information into the genome of the target cell, and thus, theoretically, the target cell's genotype is permanently modified (*1*, *2*, *5*). This is an advantage when treating hereditary and chronic disorders, but it has risks, including the potential for toxicity associated with chronic overexpression or insertional mutagenesis (for example, if the proviral DNA randomly disrupts a tumor suppressor gene or activates an oncogene). The use of retrovirus vectors is limited by the sensitivity of the vector to inactivation, by the fact that target cells must proliferate in order to integrate the proviral DNA into the genome, and by production problems associated with recombination, rearrangements, and low titers (*1*, *2*, *5*). Retrovirus vectors have been used almost entirely in ex vivo gene transfer trials.

Adenovirus vectors in current use accommodate expression cassettes up to 7.5 kb (*1*, *2*, *6*). These vectors enter the cell by means of two receptors: a specific receptor for the adenovirus fiber and $\alpha_v\beta_3$ (or $\alpha_v\beta_5$) surface integrins that serve as a receptor for the adenovirus penton (*7*) (Fig. 2). Adenovirus vectors are well suited for in vivo transfer applications because they can be produced in high titers (up to 10^{13} viral particles/ml) and they efficiently transfer genes to nonreplicating and replicating cells (*8*). The transferred genetic information remains epichromosomal, thus avoiding the risks of permanently altering the cellular genotype or of insertional mutagenesis. However, adenovirus vectors in current use evoke nonspecific inflammation and antivector cellular immunity (*9*). These responses, together with the epichromosomal position of the expression cassette, limit the duration of expression to periods ranging from weeks to months. Thus adenovirus vectors will have to be readministered periodically to maintain their persistent expression. Although it is unlikely that

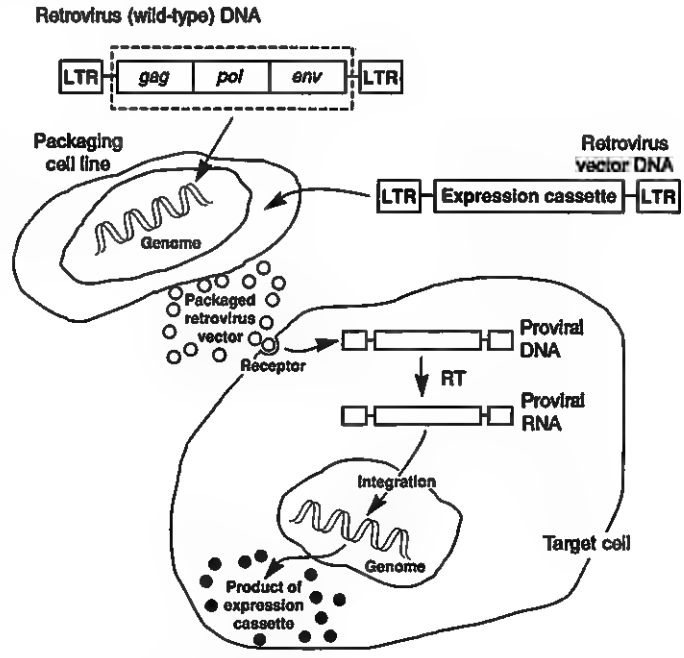

Fig. 1. Retrovirus vector design, production, and gene transfer. Retroviruses are RNA viruses that replicate through a DNA intermediate. The retrovirus vectors administered to humans all use the Maloney murine leukemia virus as the base. The *gag*, *pol*, and *env* sequences are deleted from the virus, rendering it replication-deficient. The expression cassette is inserted, and the infectious replication-deficient retrovirus is produced in a packaging cell line that contains the *gag*, *pol*, and *env* sequences that provide the proteins necessary to package the virus. The vector with its expression cassette enters the target cell via a specific receptor. In the cytoplasm, the reverse transcriptase (RT) carried by the vector converts the vector RNA into the proviral DNA that is randomly integrated into the target cell genome, where the expression cassette makes its product.

The author is professor of medicine and chief of the Division of Pulmonary and Critical Care Medicine, The New York Hospital–Cornell Medical Center, 520 East 70th Street, ST505, New York, NY 10021, USA.

repeat administration will be risky, it is not known whether antibodies directed against vector capsid proteins will limit the efficacy of repetitive administration of these vectors (9). Adenovirus vectors have been used only in in vivo human trials.

In theory, plasmid-liposome complexes have many advantages as gene transfer vectors, in that they can be used to transfer expression cassettes of essentially unlimited size, cannot replicate or recombine to form an infectious agent, and may evoke fewer inflammatory or immune responses because they lack proteins (10) (Fig. 3). The disadvantage of these vectors is that they are inefficient, requiring that thousands of plasmids be presented to the target cell in order to achieve successful gene transfer. The available data are not sufficient to determine if repetitive administration of liposomes or DNA poses safety risks. Plasmid-liposome complexes have been used only in in vivo human trials.

Expression cassettes and clinical targets. Human gene transfer studies fall into two categories: marking and therapeutic (Table 1). The marking studies use expression cassettes with bacterial antibiotic-resistant genes, which allow the genetically modified cells to be identified (Table 1). Because the marking genes have no function (other than to permit selection of the modified cells in vitro), the trials using marker genes have been designed to demonstrate the feasibility of human gene transfer, to uncover biologic principles relevant to human disease, and to evaluate safety. These trials have mostly used retrovirus vectors and have focused on malignant disorders or on human immunodeficiency virus (HIV) infection.

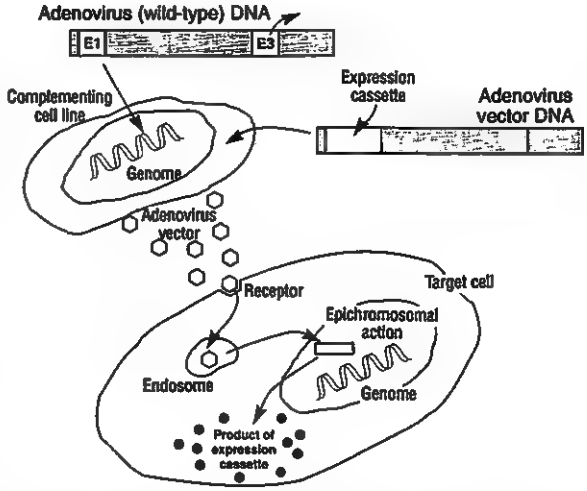

Fig. 2. Adenovirus vector design, production, and gene transfer. Adenoviruses are DNA viruses with a 36-kb genome. The wild-type adenovirus genome is divided into early (E1 to E4) and late (L1 to L5) genes. All adenovirus vectors administered to humans use adenovirus serotypes 2 or 5 as the base. The ability of the adenovirus genome to direct production of adenoviruses is dependent on sequences in E1. To produce an adenovirus vector, the E1 sequences (and E3 sequences if the space is needed) are deleted. The expression cassette is inserted, and the vector DNA is transfected into a complementing cell line with E1 sequences in its genome. The adenovirus vector with its expression cassette is E1$^-$ and thus incapable of replicating. The vector binds to the target cell through an interaction of the adenovirus fiber and penton, each to a specific receptor; moves into a cytoplasmic endosome; and breaks out and delivers its linear, double-stranded DNA genome with the expression cassette into the nucleus, where it functions in an epichromosomal fashion to direct the expression of its product.

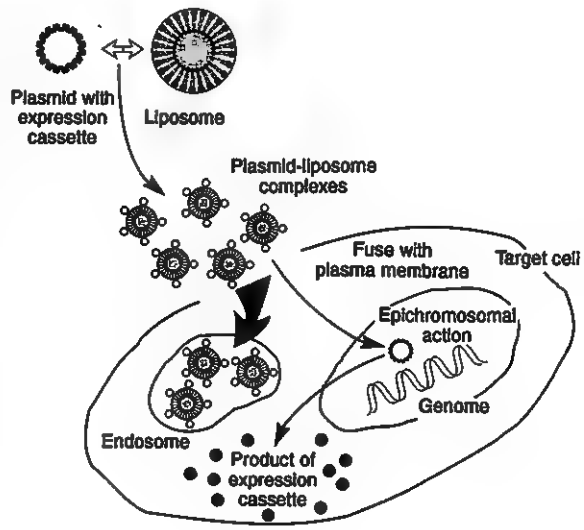

Fig. 3. Plasmid-liposome complex design and gene transfer. The liposomes used in human gene transfer trials have various compositions, but typically include synthetic cationic lipids. The positively charged liposome is complexed to the negatively charged plasmid with its expression cassette. The complexes enter the target cell by fusing with the plasma membrane. The vector does not have an inherent macromolecular structure that conveys information to enable efficient translocation of the plasmid to the nucleus. Consequently, most of the newly introduced genetic material is wasted as it is shunted to cytoplasmic organelles. When used in vivo, it is likely that most, if not all, of the plasmids that reach the nucleus function in an epichromosomal fashion.

The therapeutic trials seek to transfer expression cassettes carrying genes that will evoke biologic responses that are relevant to the treatment of human disease, and to demonstrate that this can be accomplished safely. The therapeutic studies have used retrovirus vectors, adenovirus vectors, or plasmid-liposome complexes. All of the therapeutic trials have been directed toward monogenic hereditary disorders or cancer.

What Has Really Been Accomplished?

Feasibility of gene transfer. Probably the most remarkable conclusion drawn from the human trials is that human gene transfer is indeed feasible. Although gene transfer has not been demonstrated in all recipients, most studies have shown that genes can be transferred to humans whether the strategy is ex vivo or in vivo, and that all vector types function as intended. Taken together, the evidence is overwhelming, with successful human gene transfer having been demonstrated in 28 ex vivo and 10 in vivo studies (Table 1).

In the ex vivo studies with retrovirus vectors, successful gene transfer to humans has been shown by the transfer of marker genes to various classes of T cells (*11–16*), to stem cells in blood and marrow (*16–27*), to tumor-infiltrating lymphocytes (TILs) (*11, 28, 29*), to neoplastic cells of hematopoietic lineage (*16, 17, 20, 21, 25, 26*), and to neoplastic cells derived from solid tumors (Table 1). Although there is variation among ex vivo clinical trials in the proportion of genetically marked cells recovered from the recipients, retroviral vector DNA or marker gene–derived mRNA or both have been observed in cells collected after periods ranging from several weeks to 36 months after administration.

Retrovirus vectors also have been used to transfer therapeutic genes ex vivo, with success demonstrated by the fact that the modified cells exhibit their altered phenotype in vivo for up to 36 months (Table 1). Typically, the expression cassette containing the therapeutic gene also contains an antibiotic-resistance gene, permitting the ex vivo selection of genetically modified cells recovered from the recipient. Successful gene transfer has been demonstrated in cells recovered from children with adenosine deaminase (ADA) deficiency after transfer of the normal ADA complementary DNA (cDNA) to autologous T cells, cord blood, and placental cells (*30–32*); from individuals with solid tumors after transfer of cytokine cDNAs in autologous vaccine strategies to fibroblasts, TILs, or tumor cells (*33–37*); from individuals with familial hypercholesterolemia after transfer of the low-density lipoprotein (LDL) receptor cDNA to autologous hepatocytes (*38, 39*);

from HIV$^+$ siblings after transfer of a chimeric T cell receptor cDNA to blood T cells of a twin (*40*); and from individuals with tumors who received autologous marrow transplants after transfer of the multidrug resistance 1 cDNA to autologous blood CD34$^+$ stem cells (*41*). A retrovirus vector has also been used in vivo to successfully transfer a p53 antisense cDNA to lung carcinoma cells (*42*). Finally, in a combined ex vivo–in vivo strategy for treatment of brain neoplasms, gene transfer to tumor cells has been observed after xenogeneic cells (murine fibroblasts whose genome had been modified with amphotropic packaging sequences) infected with a retrovirus vector containing an expression cassette with the herpes simplex thymidine kinase (HSTK) gene were introduced into the tumor (*43*).

In in vivo studies with adenovirus vectors, several studies have shown that direct administration of a vector containing the normal human cystic fibrosis transmembrane conductance regulator (CFTR) cDNA to the nasal or bronchial epithelium of individuals with cystic fibrosis (CF) results in transfer of the CFTR cDNA-containing expression cassette to the epithelium, where CFTR mRNA or protein is expressed for at least 9 days (*44–50*) (Table 1). Direct administration of a plasmid-liposome complex containing an expression cassette with the CFTR cDNA to the nasal epithelium of individuals with CF resulted in expression of CFTR mRNA in the epithelium (*51*). Finally, plasmid-liposome complexes have

Table 1. Summary of studies showing that transfer of genes to humans is feasible. Data shown are based on published articles and abstracts and on RAC-mandated biannual reports of principal investigators as of the RAC meeting of 8 to 9 June 1995. Abbreviations used for vector study type are RV, retrovirus; Ad, adenovirus; PL, plasmid-liposome complex; M, marker-type study; and T, therapeutic-type study. Abbreviations used for gene products are Neo^R, neomycin phosphotransferase; Hygro, hygromycin phosphotransferase; HSTK, herpes simplex thymidine kinase; ADA, adenosine deaminase; LDLR, low-density lipoprotein receptor; TNF, tumor necrosis factor α; CD4 zeta-R, chimeric T cell receptor; MDR-1, multidrug resistance 1; IL-4, interleukin 4; GM-CSF, granulocyte macrophage colony-stimulating factor; CFTR, cystic fibrosis transmembrane conductance regulator; and B7 + β_2, histocompatibility locus antigen class I–B7 + β_2 microglobulin. Except for Neo^R, Hygro, and HSTK, all genes are cDNAs. Abbreviations used for target cells are TIL, tumor-infiltrating lymphocytes; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus 1; and CTL, cytotoxic T lymphocytes. All target cells are autologous unless otherwise specified. Abbreviations used to characterize study populations are AML, acute myelogenous leukemia; CML, chronic myelogenous leukemia; ALL, acute lymphocytic leukemia; ca, carcinoma; and CF, cystic fibrosis. Under in vivo evidence of gene transfer, a plus sign indicates a report of transfer or expression (or both) of an exogenous gene in cells obtained from one or more individuals in the study; time listed is the longest time after administration that gene transfer or expression was observed.

Vector study type	Gene product	Target cells	Study population	In vivo evidence of gene transfer	Principal investigator	Reference number
RV-M	Neo^R	TIL	Melanoma	+ 2 months	Rosenberg, S. A.	(*28*)
RV-M	Neo^R	TIL	Melanoma	+ 3 months	Lotze, M. T.	(*29*)
RV-M	Neo^R	Marrow	AML	+36 months	Brenner, M. K.	(*16, 17*)
RV-M	Neo^R	Marrow	Neuroblastoma	+29 months	Brenner, M. K.	(*18*)
RV-M	Neo^R	Marrow	Neuroblastoma	+20 months	Brenner, M. K.	(*18*)
RV-M	Neo^R	Marrow	CML	+ 5 months	Deisseroth, A. B.	(*20*)
RV-M	Neo^R	Marrow	AML, ALL	+12 months	Cornetta, K.	(*21*)
RV-M	Neo^R	CD4$^+$, CD8$^+$, blood, TIL	Melanoma, renal cell ca	+	Economou, J. S.	(*11*)
RV-M	Neo^R	CD34$^+$ blood, marrow	Multiple myeloma	+18 months	Dunbar, C. E.	(*22, 23*)
RV-M	Neo^R	CD34$^+$ blood, marrow	Breast ca	+18 months	Dunbar, C. E.	(*23, 24*)
RV-M	Neo^R	Marrow	AML	+12 months	Brenner, M. K.	(*25*)
RV-M	Neo^R	Normal twin blood T cells†	Identical twins, 1 HIV$^+$	+ 4 months	Walker, R. E.	(*12*)
RV-M	Neo^R	Blood, marrow	CML	+	Deisseroth, A. B.	(*26*)
RV-M	Neo^R	CD34$^+$ blood	Metastatic ca, lymphoma	+15 days	Schuening, F. G.	(*27*)
RV-M	Neo^R	EBV-specific CTL‡	Ca, leukemia	+ 7 months	Heslop, H. E.	(*14, 15*)
RV-M	Hygro + HSTK	CD8$^+$ HIV *gag* specific, CTL§	HIV$^+$, lymphoma	+14 days	Greenberg, P.	(*13*)
RV-T	ADA	Blood T cells	ADA deficiency	+36 months	Blaese, R. M.	(*30, 31*)
RV-T	ADA	Cord blood cells	ADA deficiency	+18 months	Blaese, R. M.	(*30, 32*)
RV-T	LDLR	Hepatocytes	Familial hypercholesterolemia	+ 4 months	Wilson, J. M.	(*38, 39*)
RV-T	TNF	TIL	Melanoma	+	Rosenberg, S. A.	(*33*)
RV-T	IL-2	Tumor cells‖	Metastatic ca	+	Rosenberg, S. A.	(*36*)
RV-T	IL-2	Neuroblastoma‖	Metastatic ca	+	Brenner, M. K.	(*35*)
RV-T	CD4 zeta-R	Normal twin blood T cells†	Identical twins, 1 HIV$^+$	+ 4 months	Walker, R. E.	(*40*)
RV-T	MDR-1	Blood CD34$^+$	Breast ca	+	Deisseroth, A. B.	(*41*)
RV-T	IL-4	Fibroblasts¶	Metastatic ca	+	Lotze, M. T.	(*34*)
RV-T	GM-CSF	Melanoma‖	Melanoma	+	Dranoff, G.	(*37*)
RV-T	Anti-sense p53	Lung ca	Lung ca	+ 1 days	Roth, J. A.	(*42*)
RV-T*	HSTK	Tumor cells	Glioblastoma	+	Oldfield, E. H.	(*43*)
Ad-T	CFTR	Nasal, airway epithelium	CF	+ 9 days#	Crystal, R. G.	(*44, 45*)
Ad-T	CFTR	Nasal epithelium	CF	+	Welsh, M. J.	(*46, 47*)
Ad-T	CFTR	Nasal epithelium	CF	+	Welsh, M. J.	(*48*)
Ad-T	CFTR	Airway epithelium	CF	+ 5 days**	Wilson, J. M.	(*49*)
Ad-T	CFTR	Nasal epithelium	CF	+	Boucher, R. C.	(*50*)
PL-T	CFTR	Nasal epithelium	CF	+ 4 days	Geddes, D. M.	(*51*)
PL-T	B7 + β_2	Melanoma	Metastatic ca	+ 3 days	Nabel, G. J.	(*52*)
PL-T	B7 + β_2	Colorectal ca	Metastatic ca	+	Rubin, J. T.††	(*53, 54*)
PL-T	B7 + β_2	Renal cell ca	Metastatic ca	+	Volgelzang, N.††	(*54, 55*)
PL-T	B7 + β_2	Melanoma	Metastatic ca	+	Hersh, E.††	(*56*)

*This study used a mixed ex vivo–in vivo strategy, in which a xenogenic fibroblast cell line was modified with a retrovirus to produce an amphotropic retrovirus vector containing an expression cassette with the genes for Neo^R + HSTK, and the modified retrovirus-producing cell line was administered directly into the tumor. †Blood T cells from a normal identical twin modified with an expression cassette and then administered to an HIV$^+$ twin. ‡Allogeneic. §The HSTK gene used as a marker gene. ‖Autologous tumor cells modified with an expression cassette, lethally irradiated, and then administered as a "vaccine." ¶Autologous fibroblasts modified with an expression cassette, lethally irradiated, and then administered together with autologous, unmodified tumor cells as a "vaccine." #Messenger RNA at 9 days, vector DNA at 15 days. **A few + cells were observed at 90 days. ††Collaborative study, different institutions.

been used to transfer the human leukocyte antigen (HLA)–B7 and β2 microglobulin cDNAs directly to solid tumors in vivo, with consequent expression of the transfer cassette being seen in the tumor (*52–56*).

Relevant biologic responses. No human disease has been cured by human gene transfer, and it is not clear when this will be accomplished. However, several studies have demonstrated that therapeutic genes transferred to humans by means of retrovirus, adenovirus, and plasmid-liposome vectors can evoke biologic responses that are relevant to the gene product and to the specific disease state of the recipient (Table 2). Most of the studies demonstrating biologic efficacy have focused on monogenic hereditary disorders, where it is rational to believe that, if the normal gene product could be appropriately expressed at the relevant site, the abnormal biologic phenotype could be corrected.

Severe combined immunodeficiency–ADA deficiency is a fatal recessive disorder caused by mutations in the gene encoding ADA; these mutations cause accumulation of adenosine and 2′-deoxyadenosine, which are toxic to lymphocytes (*57*). Affected children are unable to generate normal immune responses and develop life-threatening infections. The normal ADA cDNA was transferred ex vivo with a retrovirus vector into T lymphocytes of two children with this disorder, and the modified T cells were expanded in the laboratory and periodically infused into the autologous recipients (*30, 31*). This resulted in an increase in T cell numbers and in the ADA levels in circulating T cells. The two children now have partially reconstituted immune function, as demonstrated by T cell cytokine release, cytotoxic T cell activity, isohemagglutinin production, and skin test responses to common antigens. In addition, three infants with ADA deficiency who received autologous infusions of cord blood $CD34^+$ stem cells modified ex vivo with a retrovirus vector containing the normal ADA cDNA have also shown evidence of increased numbers of blood T cells and increased ADA levels in T cells (*30, 32*). The results of the ADA studies are difficult to interpret, because none of these trials have been controlled and the recipients have also received the standard therapy of enzyme infusions with mono-methoxypolyethylene glycol-bovine ADA. Despite these caveats, these observations are consistent with the conclusion that this ex vivo gene transfer strategy evokes biologic responses that are relevant to treatment of ADA deficiency.

Familial hypercholesterolemia is a fatal disorder caused by a deficiency of LDL receptors in the liver that are secondary to mutations in the LDL receptor genes (*38, 39, 58*). The consequences are high levels of serum cholesterol and LDL cholesterol, premature atherosclerosis, and myocardial infarction. A retrovirus vector was used ex vivo to transfer the normal LDL receptor cDNA to autologous hepatocytes obtained by partial liver resection of an individual with this disorder (*38, 39*). After reinfusion of the modified hepatocytes into the liver via the portal vein, there was a reduction in LDL cholesterol and in the ratio of LDL to high-density lipoprotein over 18 months, which is consistent with the concept that the corrected cells functioned in vivo to internalize and metabolize LDL cholesterol appropriately. Like the ADA deficiency studies, this study was partially compromised because other therapies were being administered. Furthermore, the LDL receptor gene mutations were mild and could have responded to experimental variables other than the transferred gene (*58*). However, similar transfer of autologous hepatocytes modified ex vivo to other individuals with more severe mutations of the LDL receptor gene demonstrated partial correction of a variety of lipoprotein-related metabolic parameters, which is consistent with the conclusion that this gene transfer strategy did evoke a relevant response (*38*).

Cystic fibrosis is the most common lethal hereditary disorder in North America (*59*). It is caused by mutations in the CFTR gene, a gene coding for an adenosine 3′,5′-monophosphate (cAMP)–regulatable chloride channel in the apical epithelium. As a result of these mutations, the airway epithelium is deficient in CFTR function. This leads to chronic airway infection and inflammation and progressive respiratory derangement. There is compelling logic to the argument that these lung derangements could be prevented if CFTR function could be restored in these cells (*60*). It is difficult to assess CFTR function in the airway epithelium in vivo in humans, but the nasal

Table 2. Data from human gene transfer studies in which transfer of genetic material has evoked a biologic response that is relevant to the underlying disease.

Disease category	Disease	Strategy	Vector	Gene product*	Target cells	Relevant biologic response	Principal investigator	Reference number
Hereditary	ADA deficiency	Ex vivo	Retrovirus	ADA	Blood T cells and cord blood $CD34^+$ stem cells	Partial restoration of immune response	Blaese, R. M.	(*30–32*)
	Familial hypercholesterolemia	Ex vivo	Retrovirus	LDLR	Hepatocytes	Partial correction of lipid abnormalities	Wilson, J. M.	(*38, 39*)
	Cystic fibrosis	In vivo	Adenovirus	CFTR	Nasal epithelium	Partial correction of potential difference abnormalities across the nasal epithelium	Welsh, M. J. Crystal, R. G.	(*46, 47*) (*44, 62*)
	Cystic fibrosis	In vivo	Plasmid-liposome complex	CFTR	Nasal epithelium	Partial correction of potential difference abnormalities across the nasal epithelium	Geddes, D. M.	(*51*)
Acquired	Solid tumors	In vivo	Plasmid-liposome complex	HLA-B7 + β_2	Tumor cells†	Specific immune response to tumor	Nable, G. J. Rubin, J. Vogelzang, N. Hersh, E.	(*52*) (*53, 54*) (*54, 55*) (*54, 56*)
		Ex vivo	Retrovirus	IL-4	Fibroblasts‡§	Specific and nonspecific immune response to tumor	Lotze, M.	(*34*)
		Ex vivo	Retrovirus	IL-2	Neuroblastoma‡	Specific and nonspecific immune response to tumor	Brenner, M. K.	(*35*)

*ADA, adenosine deaminase deficiency; LDLR, low-density lipoprotein receptor; CFTR, cystic fibrosis transmembrane conductance regulator; HLA-B7 + β_2, histocompatibility locus antigen class I–B7 + β_2 microglobulin; IL-4, interleukin-4. †Direct administration to melanoma, colorectal carcinoma, or renal cell carcinoma. ‡Lethally irradiated, used as a "vaccine." §Combined with lethally irradiated, unmodified autologous tumor cells.

epithelium has been used as a surrogate to test the hypothesis that in vivo transfer of the normal CFTR cDNA will correct the functional consequences of CFTR deficiency (*47*, *61*). The parameters measured relate to the observation that the deficiency in CFTR causes an abnormal potential difference between the nasal epithelial surface and subcutaneous tissues. Although the nasal epithelium is not identical to the airway epithelium, two of three studies with adenovirus vectors (*44–47*, *50*, *62*) and one with plasmid-liposome complexes (*51*) have demonstrated that in vivo transfer of the CFTR cDNA to the nasal epithelium evokes a partial correction of these potential difference abnormalities for 1 to 2 weeks.

There are also studies in which human gene transfer appears to have initiated biologic responses that are relevant to therapy for an acquired disorder. These are all "tumor vaccine" studies, based on the hypothesis that exaggerated local expression of an immune-related cytokine might help activate the immune system sufficiently to recognize tumor antigens and control the growth of tumor cells. In one ex vivo study, a retrovirus vector was used to transfer the interleukin-4 (IL-4) cDNA to autologous fibroblasts (*34*). The cells were then irradiated and implanted subcutaneously in the donor together with irradiated, unmodified, autologous tumor cells. In some recipients, this evoked infiltration with $CD3^+$ T cells and tumor-specific $CD4^+$ T cells at the immunization site, as well as enhanced expression of cell adhesion molecules on capillary endothelium. In another trial, autologous neuroblastoma cells modified ex vivo with a retrovirus to contain the IL-2 cDNA were lethally irradiated and implanted subcutaneously (*35*). In some individuals, this evoked systemic augmentation of $CD16^+$ natural killer cells and tumor-specific $CD8^+$ cytotoxic T cells and eosinophilia. Finally, in four trials, in vivo plasmid-liposome complexes were used to transfer a heterologous HLA class I–B7 cDNA and the β_2 microglobulin cDNA directly to solid tumors (*52–56*). In several patients, there was evidence that the gene transfer process initiated amplification of the numbers of detectable, circulating, tumor-specific cytotoxic T cells.

Insights into human biology. Experience with marking studies has shown that human gene transfer can yield important insights into human biology by making it possible to track the fate of genetically marked cells in a recipient. For example, when stored autologous marrow is used to rescue a patient from the suppression of marrow function that complicates high-dose chemotherapy for late-stage malignancy, the individual may subsequently develop a recurrence of the malignancy. Gene transfer marking studies have helped answer the question of whether the recurrence is secondary to a residual tumor in the patient or is derived from malignant cells contaminating the reinfused banked marrow. Several studies that used an ex vivo strategy with a retrovirus vector to mark marrow cells with a neomycin resistance (neo^R) gene and then reinfused the marked marrow have demonstrated that contamination of the autologous marrow with malignant cells is common (*11*, *16–25*). These observations have led to more attention being focused on purging banked autologous marrow of contaminating neoplastic cells before they are reinfused.

There are a number of strategies being developed for the use of ex vivo gene transfer to protect autologous T cells from infection with the HIV-1. None will work, however, if autologous T cells manipulated in the laboratory and then reinfused into an HIV^+ individual have a short biologic half-life. The life-span of an autologous T cell in HIV^+ individuals has been evaluated in identical twin pairs in which one twin is HIV^+ and the other is HIV^- (*12*). A retrovirus vector was used ex vivo to transfer the neo^R gene into the T cells from the normal twin, and the genetically marked cells were then reinfused into the HIV^+ twin. Some $CD4^+$ and $CD8^+$ marked T cells (or their progeny) survived for at least 10 months, providing a baseline to allow future studies to compare the fate of T cells that have been genetically modified to prevent HIV infection.

In a strategy to prevent reactivation of Epstein-Barr virus (EBV) and the accompanying associated lymphoproliferative disease after bone marrow transplantation, allogenic EBV-specific cytotoxic T cells (CTL) were genetically marked with a retrovirus vector, and the cells were infused into individuals at risk (*15*, *16*). This preliminary study suggested that EBV-specific allogenic cells may help control EBV-associated complications of marrow transplantation, and the use of the marker genes demonstrated that the infused EBV-specific CTL persisted in the recipients for 10 weeks.

Two types of therapeutic studies support the biologic concept that minimal correction of a genotype can have significant phenotypic consequences. In the ex vivo study of retrovirus-mediated transfer of the LDL receptor cDNA into autologous hepatocytes in patients with familial hypercholesterolemia, liver biopsy several months after reinfusion of the modified hepatocytes showed that at most 5% of the total hepatocyte population expressed the normal gene in vivo (*38*, *39*, *62*). Despite this minimal correction, in some of the recipients there were changes in LDL-related parameters that suggested LDL receptor function in the liver had been partially restored. Partial phenotypic correction has also been observed in most of the trials of adenovirus- and plasmid-liposome complex–mediated in vivo transfer of the CFTR cDNA to the nasal epithelium in CF, even though the amount of gene transfer and expression has been limited to a small fraction of the target cells (*44–47*, *50*, *51*, *62*).

Finally, when adenovirus vectors are administered to experimental animals, the animals quickly develop circulating neutralizing antibodies directed against the vector (*9*). In two studies of administration of adenovirus vectors to the airways of individuals with CF, no circulating neutralizing antibodies were detected (*44*, *45*, *49*). This is an important observation, because the expression cassette delivered by adenovirus vectors remains epichromosomal, and thus the vector will have to be readministered as its expression wanes. Although it is possible that there are local antibodies to the vectors in these individuals (*9*), the lack of a systemic immune response to such an antigen load is encouraging in that it suggests that antibodies to vectors may not be a major factor limiting persistent vector expression in humans when the lung is repeatedly dosed (*64*).

Safety of gene transfer. The theoretical safety concerns regarding human gene transfer are not trivial. For the individual recipient, there is the possibility of vector-induced inflammation and immune responses, of complementation of replication-deficient vectors leading to overwhelming viral infection, and (for the retrovirus vectors) of insertional mutagenesis. There are also theoretical issues that are important to society, including concerns about modifying the human germ line and about protecting the environment from new infectious agents generated from gene transfer vectors carrying expression cassettes with powerful biologic functions.

There have been adverse events in the human gene transfer trials, including inflammation induced by airway administration of adenovirus vectors (*44–50*, *65*) and by administration to the central nervous system of a xenogenic producer cell line releasing a retrovirus vector (*43*, *66*). However, compared with the total numbers of individuals undergoing gene transfer, adverse events have been rare and have been related mostly to the dose and the manner in which the vectors were administered. Shedding of viral vectors in the in vivo trials was very uncommon and was limited in extent and time (*42*, *44–50*, *65*). No novel infectious agents generated from recombination of the transferred genome and the host genome or other genetic information have been detected, nor has any replication-competent virus related to the vector. Cells modified ex vivo with retrovirus vectors have been infused repeti-

tively without adverse effects (*13*, *30*, *31*, *35*), adenovirus vectors have been administered repetitively in vivo to the nasal (*48*) and bronchial epithelium safely (*64*, *67*), and plasmid-liposome complexes have been administered repetitively to tumors in vivo without complications (*52–56*). Finally, human gene transfer has not been implicated in initiating malignancy, although the numbers of recipients and time of observation will have to be much greater to allow definitive conclusions regarding this issue.

What Are the Obstacles to Successful Human Gene Transfer?

With the successes of the human gene transfer trials have come the sobering realities of the drug development process. Some of the problems are generic for the field, and some are specific for each vector.

Inconsistent results. All of the human gene transfer studies have been plagued by inconsistent results, the bases of which are unclear. For example, in the two children with ADA deficiency receiving intermittent infusions of autologous T cells modified ex vivo with the normal ADA cDNA, the resulting proportion of ADA^+ circulating T cells has varied from 0.1 to 60% (*30*, *31*). In the CF trials, there is evidence that adenovirus vectors and plasmid-liposome complexes can transfer the normal CFTR cDNA to the respiratory epithelium, but expression is observed in at most 5% of the target cells and is not seen in all recipients (*44–51*, *65*). Further, an appropriate biologic response to gene transfer (correction of the abnormal potential difference across the nasal epithelium) has been observed in some patients in most, but not all, of the studies of CFTR cDNA transfer (*44–47*, *50*, *51*, *62*). In most of the ex vivo marrow-marking trials, successful gene transfer is observed intermittently (Table 1).

Humans are not simply large mice. There have been several surprise examples, in which predictions from gene transfer studies in experimental animals have not been borne out in human safety and efficacy trials. In tumor vaccine studies intended to evoke a tumor-directed immune response, there is no convincing evidence (other than anecdotal case reports) that tumors regress, despite the promising observations in experimental animals (*34*, *37*, *52–56*). It has also become apparent that studies in experimental animals may not necessarily predict the toxicology of vectors in humans. In one patient with CF in whom 2×10^9 plaque-forming units of an adenovirus vector containing the CFTR cDNA were administered to the lung, a transient local and systemic inflammatory syndrome was evoked, despite the fact that no clinically apparent toxicity was observed in rodents and nonhuman primates receiving 1000-fold greater doses by the same route (*45*). Likewise, in an ex vivo–in vivo strategy to treat glioblastoma, transfer of xenogenic retrovirus-producing cells to the tumor was accomplished without significant adverse effects in experimental animals, but the human studies have been associated with central nervous system toxicity related to transfer of the cell line to the tumor (*43*, *66*).

Production problems. There are significant hurdles in vector production that must be overcome before large clinical trials can be initiated. Generation of replication-competent virus is observed in production of clinical-grade retrovirus and adenovirus vectors; and lack of reproducibility, aggregation, and contamination with endotoxin complicate the production of clinical-grade plasmid-liposome complexes (*68*).

The perfect vector. The ideal gene transfer vector would be capable of efficiently delivering an expression cassette carrying one or more genes of the size needed for the clinical application. The vector would be specific for its target, not recognized by the immune system, stable and easy to reproducibly produce, and could be purified in large quantities at high concentrations. It would not induce inflammation and would be safe for the recipient and the environment. Finally, it would express the gene (or genes) it carries for as long as required in an appropriately regulated fashion (*69*).

This ideal vector is conceptually impractical, because the human applications of gene transfer are broad, and the ideal vector will likely be different for each application. Clinical experience to date suggests that retrovirus, adenovirus, and plasmid-liposome vectors all need refinement, but each is relatively well suited for the clinical targets at which they have been directed. Further, the technology is now available to create designer vectors that can be optimized for each application. Among the design hurdles for all vectors are the need to increase the efficiency of gene transfer, to increase target specificity and to enable the transferred gene to be regulated. Reproducible production of large amounts of pure vector is a hurdle for all classes of vectors. Some of the vector-specific hurdles are reduction of the risk for insertional mutagenesis in retrovirus vectors, minimization of the amount of immunity and inflammation evoked by the adenovirus vectors, and enhancement of the translocation of the gene to the nucleus for the plasmid-liposome complexes.

There is considerable interest in developing new vectors, but there is controversy as to which vector class is most likely to succeed, particularly for use in in vivo applications. There are two philosophical camps in vector design: viral and nonviral. The viral proponents believe that the most efficient means to deliver an expression cassette in vivo is to package it in a replication-deficient recombinant virus. The logic supporting this approach is the knowledge that viruses are masterful at reproducing themselves, and thus have evolved strategies to efficiently express their genetic information in the cells they infect. The nonviral proponents concede this argument but believe that the redundant anti-immune and inflammatory host defenses against viruses may be a risk to recipients, will limit the duration of expression as the infected cells are recognized by the immune system, and may hinder the efficacy of repeat administration of the vectors. Thus, nonviral vector aficionados believe it is rational to start from scratch to design safe, efficient, gene transfer strategies. In contrast, the viral camp believes that it is best to start with something that works but then to circumvent the replication, immune, and inflammation risks inherent in their use by appropriate vector design. It is most likely that these philosophical differences will eventually disappear as new classes of vectors are designed that incorporate features of viral and nonviral vectors, as dictated by specific clinical applications.

Future Prospects

None of the drug development problems facing human gene transfer are insurmountable, but each will take time to solve. However, the logic underlying the potential usefulness of human gene transfer is compelling; and put in a context in which the human genome project will provide 80,000 to 100,000 human genes that could be used in expression cassettes for human gene transfer, the potential impact of this technology for innovative therapies and increased understanding of human biology is enormous.

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stitutes of Health, Suite 323, 6006 Executive Boulevard, MSC 7052, Bethesda, MD 20892–7052, USA. Human data in this review are derived from published articles and abstracts and from the December 1994 and June 1995 RAC investigator reports. Because the RAC reports are mandated, frequently updated, and public, they are an accurate gauge of the status of the field, although they are not peer-reviewed. Since the first human trial was begun in 1989, there has been an explosion of interest in human gene transfer. In the United States alone, more than 100 human gene transfer protocols have been approved by the RAC, and 697 individuals have participated in human gene transfer trials under RAC-approved protocols (summary data, *RAC Report*, June 1995).
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The Nematode *Caenorhabditis elegans* and Its Genome

Jonathan Hodgkin, Ronald H. A. Plasterk, Robert H. Waterston

Over the past two decades, the small soil nematode *Caenorhabditis elegans* has become established as a major model system for the study of a great variety of problems in biology and medicine. One of its most significant advantages is its simplicity, both in anatomy and in genomic organization. The entire haploid genetic content amounts to 100 million base pairs of DNA, about 1/30 the size of the human value. As a result, *C. elegans* has also provided a pilot system for the construction of physical maps of larger animal and plant genomes, and subsequently for the complete sequencing of those genomes. By mid-1995, approximately one-fifth of the complete DNA sequence of this animal had been determined. *Caenorhabditis elegans* provides a test bed not only for the development and application of mapping and sequencing technologies, but also for the interpretation and use of complete sequence information. This article reviews the progress so far toward a realizable goal—the total description of the genome of a simple animal.

Caenorhabditis elegans has many attractive features as an experimental system (*1*). The life cycle is simple and rapid, with a 3-day generation time, and populations can be grown with ease on agar plates or in liquid, usually by using *Escherichia coli* as a food source. These populations normally consist of only self-fertilizing hermaphrodites, but cross-fertilization is also possible, with the male sexual form. The option of reproduction by either selfing or crossing leads to very convenient genetics so that mutants can readily be generated, propagated, and analyzed (*2*). A simple freezing protocol permits stable storage of all strains, which retain viability indefinitely in the frozen state.

The animal, about 1 mm long when fully grown, is completely transparent at all stages of development. Both development and anatomy are essentially invariant among wild-type individuals. At maturity, all adult hermaphrodites contain 959 somatic nuclei and fewer than 2000 germ cell nuclei. Despite its low cell number, C. *elegans* has fully differentiated tissues corresponding to those of more complicated animals. The transparency and rapid development allow direct examination of cell division and differentiation in living animals with Nomarski microscopy. The small size of the animal also permits reconstruction of the entire anatomy at the ultrastructural level with serial section electron microscopy. However, the

J. Hodgkin is in the Medical Research Council Laboratory of Molecular Biology, Cambridge, CB2 2QH, UK. R. H. A. Plasterk is in the Netherlands Cancer Institute, Division of Molecular Biology, Plesmanlaan 121, 1066 CX Amsterdam, Netherlands. R. H. Waterston is in the Department of Genetics and Genome Sequencing Center, Washington University School of Medicine, St. Louis, MO 63110, USA.

small size does lead to some concomitant disadvantages, as it largely precludes certain experimental approaches, such as tissue transplantation and electrophysiology.

Throughout research on C. *elegans* there has been an emphasis on complete description, both as an end in itself and also to provide the groundwork for experimental studies. The combination of simplicity, lineage tracing, cell identification, and ultrastructural reconstruction has led to a number of landmark accomplishments. A total description of the invariant cell lineage, from the single cell of the fertilized egg to the thousand-odd differentiated cells of the adult, was completed in 1983 (3). Accompanying this was a "parts list," enumerating all the identified epithelia, muscles, nerves, intestinal cells, and other cell types that make up the entire anatomy of the adult. All of these parts have been described and reconstructed from serial electron micrographs (EMs). The EM technique was also used for the complete description of the nervous system: The anatomy and connectivity of all 302 adult neurons, as well as the 7600 synaptic junctions they make, were reported in 1986 (4).

In parallel, experimental approaches to the C. *elegans* system have been directed at comprehensive analysis. Systematic studies of cell ablation, using a laser microbeam, have been pursued to test functions and regulation within the nervous system and the embryo (3). Large-scale hunts for mutants have been carried out, and this mutational approach has often been directed at saturating for certain mutant classes, with the intention of identifying all genes involved in particular developmental or behavioral processes. Other screens have been directed at identifying all essential genes located in defined genetic intervals. The number of genes defined by mutations has expanded from the initial set of 103 reported by Brenner (2) in 1974, to approximately 1400 in 1995. Recombinational mapping of these genes defined six linkage groups (five autosomes and an X chromosome), which are roughly equal in size and correspond to the six cytologically visible chromosomes.

Early on, measurements of DNA content and complexity indicated that the genome of C. *elegans* is very small, approaching the apparent limit for a respectably differentiated metazoan animal. This property has loomed ever larger in the investigation of C. *elegans*, first in the construction of a complete physical map, and now in the project to determine the complete DNA sequence.

The Physical Map

Systematic assembly of a physical map of the genome was begun in 1984 (5). The initial program made use of a rapid "fingerprinting" technique to identify matches between cloned pieces of DNA in the 10- to 50-kb size range. Several thousand randomly chosen cosmid clones were fingerprinted and assembled in contigs, which are overlapped sets of cosmids that extend from 50 to several hundred kilobases in length. A variety of cosmid vectors and cloning methods were explored at later stages, in the hope of maximizing coverage. Ultimately, more than 17,000 cosmids were analyzed. This phase of the project, known as the thousand islands plan, led to coverage of ~80% of the genome, with cosmids assembled into about 700 island contigs. Many of these could be assigned to precise genetic map locations as a result of the independent cloning of specific genes and also by in situ hybridization.

With time, the cosmid approach began to yield diminishing returns in terms of improving coverage and linking up contigs. The advent of yeast artificial chromosomes (YACs) provided an ideal solution to this problem (6). The YAC clones of C. *elegans* DNA have been largely reliable, exhibiting less instability and distinctly less chimerism than those derived from mammalian genomes. Systematic hybridization between YACs and selected cosmids led to the assembly of an almost complete YAC map and to the assignment of almost all cosmids to specific YACs. Ordered arrays of YAC clones on filters, known familiarly as polytene filters, are now generally available. Hybridization of any clone to these filters permits its localization to a resolution of about 100 kb. A set of cosmids corresponding to this location can then be tested, yielding a precise genomic position. All clones generated by the physical mapping program are freely available on request.

The present state of the physical map can be assessed at two levels: the YAC map and the cosmid map. In terms of YACs, the genome is extremely well covered in that there remain only seven gaps in the map, and there is reason to believe that some of these gaps are small. The X chromosome map is now complete, apart from telomeric regions, and consists of a single YAC contig of 18,000 kb. Complete coverage of one of the five autosomes has also been achieved. The reasons for the residual gaps in the autosomal map are not known at present.

A different picture is seen at the level of cosmid coverage, which is dense but far from continuous. On each chromosome there are 50 to 120 regions that are spanned by YACs but not by cosmids; in all, there are a total of about 520 such gaps. The average size of each cosmid contig is therefore ~200 kb, and the longest stretch covered by overlapping cosmids is less than 2000 kb. It is clear, both from statistical arguments and from work with specific regions, that these gaps represent parts of the genome that are difficult to clone in prokaryotic vectors, although they can readily be propagated in YACs. In some cases, the cloning problems can be overcome by using vectors with smaller inserts, or bacterial hosts with different recombinational properties: These may permit isolation of clones that cannot be obtained as cosmids, and consequently lead to gap closure (7).

Fortunately, it appears likely that many of the difficult regions of the genome, where cosmid coverage is poor, are also regions containing few genes. For example, one stretch of over 1100 kb on chromosome IV is defined only by YAC clones. No cosmids or known genes are located in this domain, which may contain only heterochromatin, or the C. *elegans* equivalent. These gene-poor regions will not be ignored, but they will be among the last parts of the genome to be subjected to systematic sequencing.

Linking Physical and Genetic Maps

The initial motive for generating such an extensive physical map was to facilitate the positional cloning of known genes. The physical map has indeed been enormously useful in this regard. Work from many laboratories, using a variety of techniques, has led to the tight correlation between the genetic and physical maps of this organism. Over most of the genome, recombinational mapping can now be used to assign a gene to a physical interval of 200 kb or less.

The C. *elegans* transposable element Tc1, a 1.6-kb element belonging to the Tc1-mariner class, has played several important roles in linking the physical and genetic maps (8). First, transposon tagging of many genes led to their direct cloning and location on the physical map. Second, restriction fragment length polymorphisms (RFLPs) generated by Tc1 have provided numerous useful landmarks. The standard laboratory strain of C. *elegans* (Bristol) has about 30 copies of Tc1, but there exist other cross-fertile strains with up to 500 copies, distributed apparently at random across the entire genome. Many of these have been cloned, and an increasing number of these have also been used to generate sequence-tagged sites, which provide additional mapping tools for polymerase chain reaction (PCR)–based mapping strategies (9). In addition to the polymorphisms generated by Tc1, additional RFLPs between the Bristol strain and other natural isolates are readily identified, at a frequency of one per cosmid or better. These can be used to provide very tight mapping resolution, should this be necessary.

Improving the correspondence between the maps has been significantly aided by the

development of the C. *elegans* database ACeDB, which provides a convenient interactive display of genetic and physical maps, plus underlying genomic and complementary DNA (cDNA) sequences, and much else besides, acting as a universal repository for all information pertaining to this organism (*10*). The correlation between the physical and genetic maps has also produced valuable information about the general organization of the genome, such as demonstrations of long-range variation in gene density and recombination (*11*). The variations in gene density can also be seen in the mapping of cDNA clones, illustrated in the accompanying wall chart.

Sequencing

The current strategy for sequencing the C. *elegans* genome is mainly based on cosmids, because this is the most cost-effective way to proceed during this phase of the project (*12*). The dense physical map permits an optimal choice of minimally overlapped cosmids. Random subclones are generated from each selected cosmid (a "shotgun" approach) and sequenced automatically, giving initial coverage of six- to eightfold for each 35- to 45-kb cosmid. Sequencing reads on each subclone generally yield more than 400 base pairs (bp) of useful data. The shotgun phase is followed by assembly and more dedicated finishing with directed reads, using oligonucleotide primers synthesized for the purpose, in order to fill gaps, to achieve double-stranded coverage, and to complete the linking of contigs. Finished sequence is currently being generated at a total cost of less than 50 cents per base, with an accuracy of ~99.99%.

This phase of the project, which will extend over most of the next 2 years, should provide most of the coding and regulatory information in the genome. However, the cosmid gaps discussed above will present an increasing problem as the project approaches completion. It is probable that further progress will depend on sequencing subclones prepared directly from YACs. This involves additional technical problems but is already feasible. The main difficulty is the unavoidable contamination of purified YACs by substantial amounts of DNA from the yeast host, leading to much wasted time in sequencing and assembling irrelevant yeast sequences. However, this difficulty should be eliminated at the end of 1995, which is the target date for the completion of the complete sequence of *Saccharomyces cerevisiae* (*13*). It will then become possible to discard instantly all sequencing reads that are recognizable as yeast DNA and focus exclusively on the C. *elegans* DNA.

The combination of efficient cosmid and YAC sequencing should yield at least 98% of the C. *elegans* genome by the end of 1998. No radical changes in technology are needed.

Interpretation of Sequence

A completed sequence is subjected to a variety of analyses to detect genes and other features. The most important of these analyses is the GENEFINDER program, which uses properties such as codon usage and splice recognition sequences to predict the coding portions of genes (*14*). Many C. *elegans* introns are small, and splice site consensuses are usually well conserved, so the prediction of exons and gene boundaries seems to be easier and more successful than in larger genomes. However, GENEFINDER is not infallible, nor can it predict alternatively spliced transcripts. An essential source of additional information has been the sequencing of numerous cDNA clones, which confirm exon boundaries predicted by GENEFINDER and also reveal splice variants. Systematic end-sequencing of cDNA clones has been pursued on a large scale (*15*), so by mid-1995 approximately 3500 genes were represented, corresponding to about one-quarter of all the transcribed sequences in the animal. Many of these expressed sequence tags (ESTs) can be immediately assigned to the parts of the genome that have been completely sequenced. Many others have been assigned to specific locations on the physical map, by hybridization to the gridded polytene filters of YAC clones.

Exploration of the sequence of C. *elegans* is an open-ended enterprise at this point, and investigations have hardly begun. Many questions that could never have been answered previously can now be directly addressed. For example, what is the total number of genes in this organism? What are the major gene families? How many genes contain recognizable similarities?

The question of gene number can already be answered fairly precisely with both the predictions from genomic sequence and the distribution of cDNAs: currently the best estimate is 13,100 genes. This is a surprisingly large number, given earlier estimates of fewer than 3000 essential genes (*2*). It suggests that the majority of genes are to some extent redundant or else have subtle functions, perhaps such as those that are significant in the natural ecology of C. *elegans* but not apparent under laboratory conditions.

It seems that we are ignorant about what most of the genome is doing, even in this relatively simple organism. This ignorance is underlined by the fact that more than half of the 3000 genes so far sequenced have no significant similarity to proteins in current databases. We have few clues as to the functions of such genes, a situation that is both challenging and exciting. However, some improvement in this state of affairs is likely to occur as more and more sequence is generated, because genes that currently appear unique may become identifiable as diverged members of known gene families. Information from many organisms can increasingly be used to identify families and ancient conserved regions (*16*).

Already much that is intriguing or exotic has emerged from the millions of base pairs of sequence, such as giant predicted proteins, genes within introns, and clustered genes of unknown function. Some of these features are illustrated in the wall chart: for example, on cosmid F10F2, four related unidentified genes nestle within the largest intron of a recognizable enzyme gene. As shown in Fig. 1, most of cosmid K07E12 seems to be used for encoding a single vast protein that has some similarity to cell adhesion molecules. The expected transcript size for K07E12.1 is 39 kb, corresponding to a predicted protein of 1400 kD.

One notable aspect of the C. *elegans* genome is that ~25% of the genes are organized in polycistronic units of two or more members (*17*). One such example, shown on the wall chart, is cosmid ZK637 which has three adjacent genes that are known to be cotranscribed. The primary transcripts from these units are processed into single-gene transcripts by transplicing to the short RNA splice leaders SL2 or SL1. In some cases, the genes within such "operons" have related functions. In others (like ZK637) there is no evidence of such a connection, but the constituent genes are presumably coexpressed.

Exploitation of Sequence: *C. elegans* as a Test Tube

There are many ways in which the basic data provided by the sequencing consortium can be used and extended. Systematic analyses of the thousands of predicted genes are already in progress. These investigations can be divided into expression studies and functional studies.

Expression patterns for cloned genes can be determined in a number of ways, as illustrated in the wall chart. Ideally, complete cellular and developmental profiles for both transcripts (by in situ hybridization) and protein products (by immunofluorescence) can be obtained. More realistically, much information can be obtained with little effort by carrying out in situ hybridization experiments with many different cDNA clones, leading to the identification of transcripts with distinctive abundance or tissue distribution. A more focused, gene-by-gene transgenic approach is also feasible by using the sequence information to con-

struct fusions between promoter regions and reporter genes such as *lacZ* or the gene for green fluorescent protein (GFP). These fusions can then be tested in transgenic animals, providing data on spatial and temporal expression for each tested gene (*18*). GFP constructs are especially useful in *C. elegans* because the animal is transparent and the patterns of expression can be examined directly in living animals (*19*). Such expression studies are informative and often suggestive as to what each gene is doing, but there is no substitute for more direct functional assays. For this, some kind of genetic approach is essential.

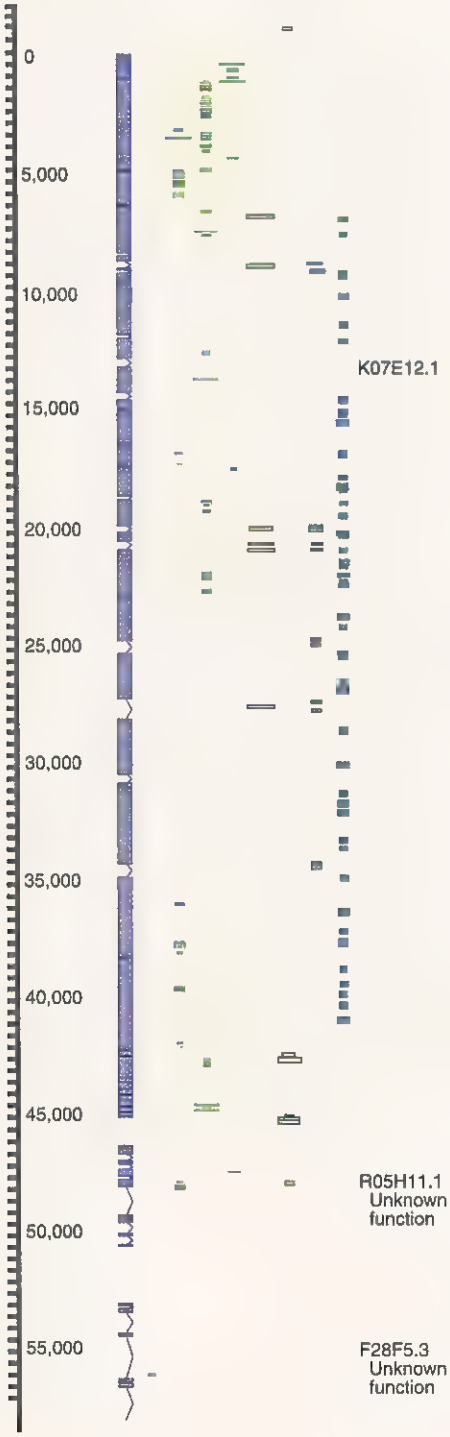

Fig. 1. The genomic region including cosmid K07E12, illustrated with a modified form of the standard ACeDB display (*10*). The scale bar at the left indicates the number of base pairs; next to this is the predicted exon organization (in purple) for three genes (K07E12.1, R05H11.1, and F28F5.3), all transcribed in the same direction. Further to the right are shown significant database matches in different reading frames (three columns), followed by columns indicating the location of repeated sequence families (wide yellow bars) and matches to *C. elegans* cDNA clones (narrower yellow bars). The two rightmost columns (blue-green bars) mark inverted and tandem repeats in the DNA sequence. The giant protein (1400 kD) predicted for K07E12.1 is extensively repetitious and has some sequence similarity to mammalian cell adhesion molecules, but is otherwise novel.

Many biologists working on vertebrate systems are becoming aware of *C. elegans* for the first time because of the identification of a nematode homolog for the gene they happen to be studying. Sometimes the homology will first be detected as partial sequence from a cDNA clone, but increasingly there will be complete genomic information available. What next? First, it is an immediate and automatic bonus to be provided with the entire gene sequence of a *C. elegans* homolog for a vertebrate gene. Sequence comparisons will pinpoint conserved and therefore important protein domains, which may not otherwise be obvious. These conserved regions can then be used to search for other related genes in a more systematic way. Other features, such as intron organization and promoter structure, may also be informative. Second, it will be possible to explore the function of the gene of interest in *C. elegans* with both expression studies and functional tests and by using the power of genetic approaches.

In some cases, mutations may have already been identified in the nematode homolog, as a result of the extensive hunts for mutants carried out in the past. As the correlation of the genetic and physical maps is refined, it is increasingly feasible to associate candidate genes with known mutants. The range of mutant phenotypes that are being studied in *C. elegans* continues to expand, and the characterization of these phenotypes has become steadily more sophisticated. In particular, there is a very large available repertoire of mutations affecting neuronal functions and behavior, a few of which are illustrated in the wall chart. About 100 genes affecting the operation of the locomotory nervous system have been defined by mutation (these are called *unc* genes, for "uncoordinated"), as well as dozens of others affecting sensory functions (chemotaxis, thermotaxis, olfaction, and mechanoreception), specific behaviors (eating, defecation, copulation, and egg-laying), neuronal plasticity, resistance to neuroactive drugs, and others, to a total of about 350 genes at present. As in other animals, a large part of the genome seems to be devoted to neurobiology, and behavior often provides a sensitive assay for subtle alterations in the normal functions of an animal.

Even if no mutations currently exist, a variety of techniques can be used for functional experimentation on an identified gene. These include overexpression, interference by antisense methods, and above all, gene disruption. Both overexpression and antisense methods entail construction of transgenic animals, which are generated by microinjection of cloned DNA (*20*). Such DNA does not normally integrate into the chromosomes but instead forms a multiple copy extrachromosomal array, which can be transmitted fairly stably to offspring. Sometimes the increased copy number of genes in the array is enough to cause a phenotypic effect by itself. Alternatively, expression levels can be boosted by fusing the gene of interest to an inducible promoter, such as a heat-shock promoter.

A more desirable goal is to disrupt the *C. elegans* homolog with procedures that are becoming steadily more streamlined. Targeted gene replacement is not currently feasible; instead, disruption is usually achieved by a two-step process of transposon insertion followed by imprecise excision of the transposon (*21*). Inserts of Tc1 in a gene of interest can be detected by a PCR method, screening DNA samples from a frozen mutant bank. Once such an insertion has been detected, the corresponding population can be thawed, subdivided, and retested. The combined process of PCR testing and sib-selection usually leads rapidly to the isolation of a single worm carrying the desired Tc1 insertion. Sometimes a Tc1 insertion is sufficient to eliminate or greatly reduce gene function [for example, in the case of the gene *goa-1* which encodes one subunit of a heterotrimeric guanosine triphosphate–binding protein (G protein) (*22*)], but often the transposon is located in an intron and has little effect on gene expression. It is then necessary to isolate a derivative mutant line in which the transposon has imprecisely excised, by means of a second round of PCR testing and sib-selection. Imprecise excision usually deletes several kilobases of flanking DNA and thereby creates a null mutation. Some examples of successful isolation of Tc1 inserts are listed in Table 1, to illustrate the range of genes to which this technique has been applied (*23*).

Once mutation or disruption of the nematode gene has been achieved, the resulting phenotype can be examined in detail, and a whole panoply of genetic tech-

Table 1. Reverse genetics: some *C. elegans* genes interrupted by Tc1 insertion.

Gene	Description
apl-1	Amyloid precursor related
cah-1	Adenylate cyclase–associated protein
cct-1	Cytoplasmic chaperone family
cdc-42	Cell cycle protein
cdh-3	Cadherin (cell adhesion molecule)
cdk-5	Cell cycle kinase
ceh-13	Homeobox (labial)
cey-1	Y-box (DNA or RNA binding)
cpr-6	Cathepsin protease
elt-2	GATA transcription factor
fkh-1	Fork-head–related transcription factor
flp-1	Neuropeptide precursor
ges-2	Intestinal carboxylesterase
goa-1	G protein (G_o, alpha subunit)
gpa-2	G protein (G, alpha subunit)
gpb-1	G protein (G, beta subunit)
hlh-3	Helix-loop-helix transcription factor
nhr-1	Nuclear hormone receptor family
odc-1	Ornithine decarboxylase
pes-9	Patterned expression site
pgp-3	P-glycoprotein (multiple-drug resistance)
prk-1	Pim-1 oncoprotein homolog
sod-2	Superoxide dismutase
ssb-1	Single-stranded DNA binding factor
tkr-1	Tachykinin receptor family

niques can be brought into play. Vertebrate homologs and engineered variants of the gene can be tested for function, permitting rapid structure-function correlations. Interactions with mutations in other genes can be explored by construction of double mutants. Screens for genetic modifiers (suppressors or enhancers) can also be carried out. A particular advantage of the C. *elegans* system is that the animal is a diploid which normally reproduces by self-fertilization, so both dominant and recessive modifiers can be selected or screened for. Once modifiers have been identified, they in turn can be mapped and analyzed at the sequence level. In this way, one can expect to identify other interacting genes and elucidate whole pathways. The power of this kind of approach has become well known; for example, in the dissection of *ras*-dependent signaling (*24*).

Prospects

Much current work in molecular biology is constrained by the substantial amount of work needed to clone and sequence a DNA segment. For C. *elegans*, we will soon enter a period in which knowledge of the complete genome sequence can be assumed. This will alter the choice of experimental strategies. Many experiments that are presently very laborious to carry out in vivo or in vitro will become simple and easy to perform "in silico" (by computer), by analyzing the many megabases of stored sequence.

It is reasonable to expect that the complete genome sequence of C. *elegans* will provide, in some sense, the basic formula for constructing a multicellular animal, in much the same way as the complete sequence of *S. cerevisiae* will reveal the basic ingredients for making and maintaining a eukaryotic cell. The phylogenetic position of C. *elegans* is convenient in this regard, because it appears that nematodes diverged at an early point from the rest of the metazoan radiation. Consequently, they provide a universal out-group for the rest of the animal kingdom (*25*). What this means is that if a gene can be identified both in C. *elegans* and in any other kind of animal, whether it be vertebrate, insect, or mollusk, then it must also have been present in the common ancestor of all animals.

With time, it should therefore become clear how much of the C. *elegans* genome is devoted to this basic animal construction kit and how much is associated with specializations that are unique to the phylum Nematoda, or to C. *elegans* itself. For example, the compactness of this genome may be correlated with idiosyncratic features such as the operons, which seem to be absent from larger genomes. Other molecular or biological properties may turn out to be unique to nematodes in general, but absent from other animal groups. Such properties will also be valuable to discern, because nematodes are a large and important group of animals in their own right. Many nematode species have considerable medical or economic significance, as agents of disease and major agricultural pests.

However, it is already clear that a large part of the genome is doing universal things, so much of what is learned from C. *elegans* will also apply to all multicellular organisms. Finally, there is an appealing novelty to the prospect of a complete sequence for such a thoroughly studied animal. How much of the genome will be comprehensible, by arguments from homology or functional experimentation, and how much will remain mysterious? Only by arriving at a complete sequence will we be able to test the limits of our understanding, and perhaps see what questions to ask next.

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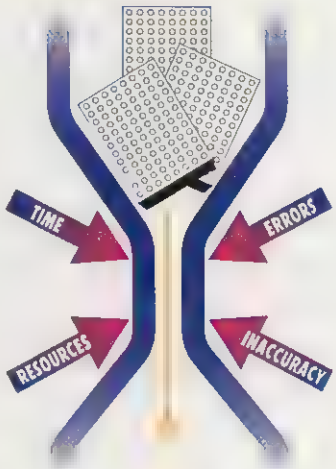

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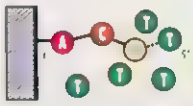

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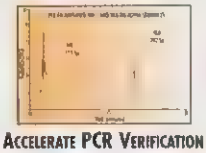

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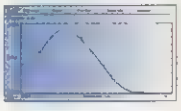

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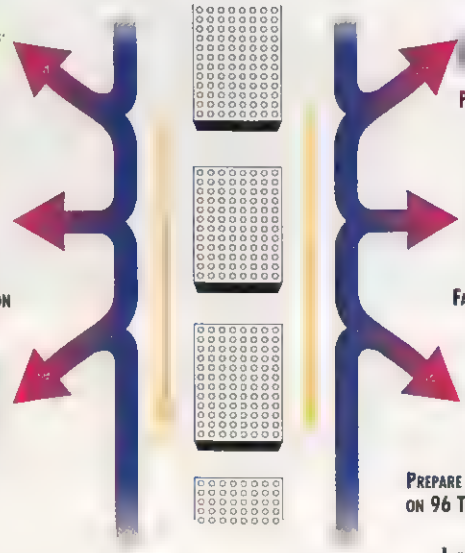

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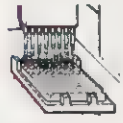

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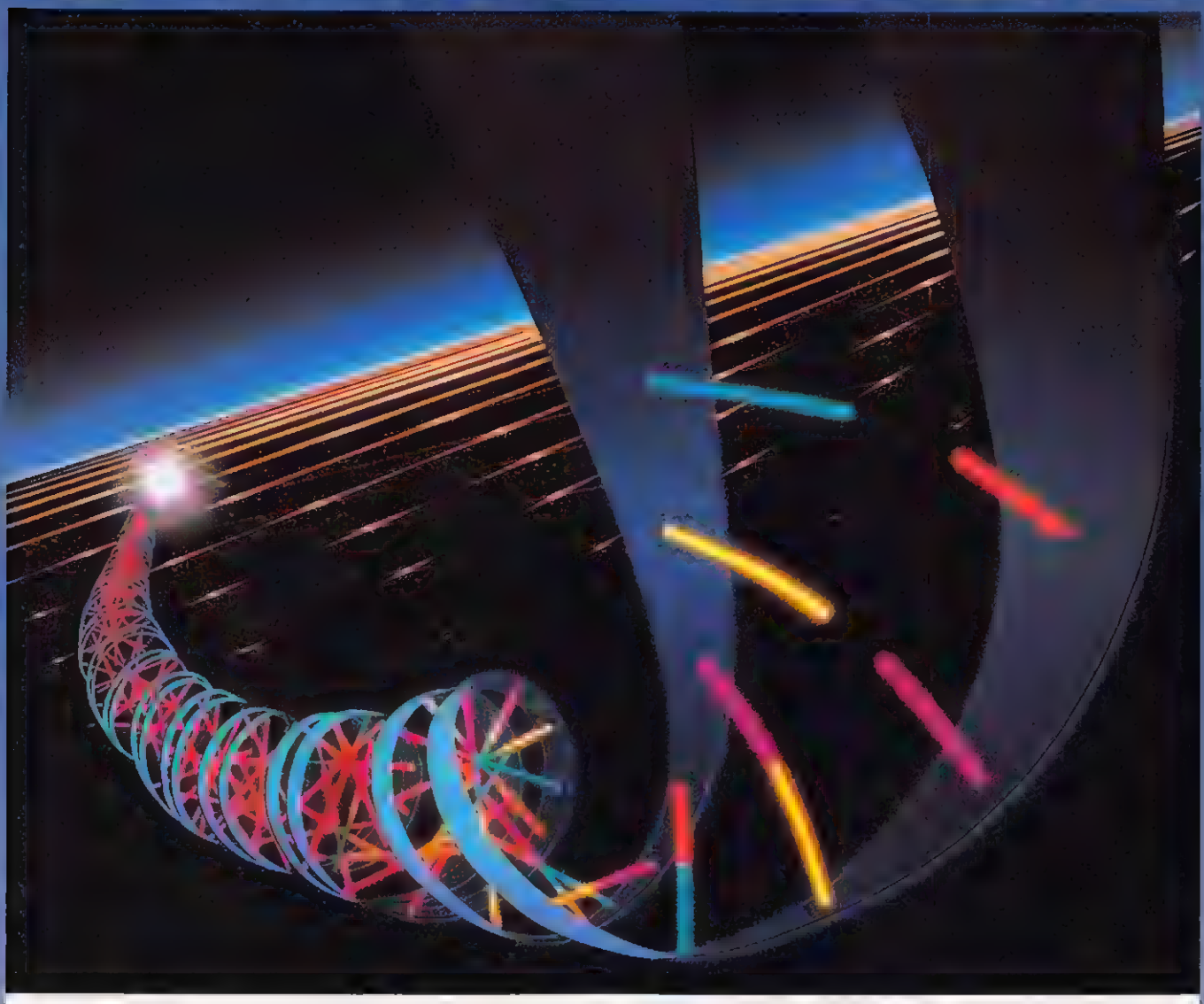

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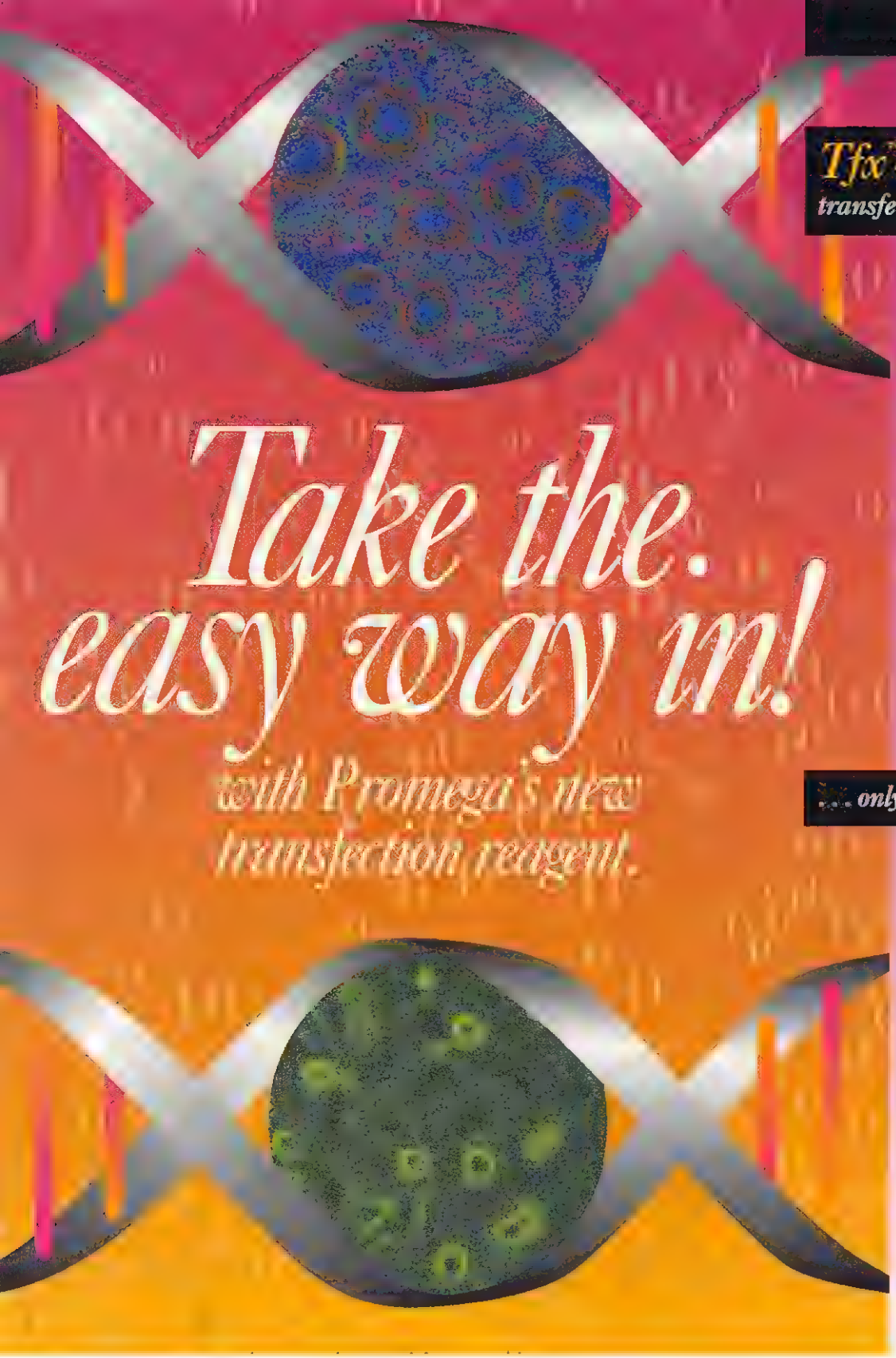

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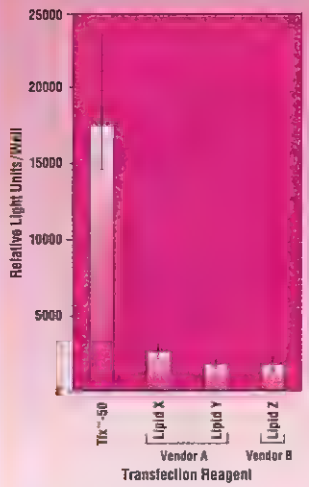

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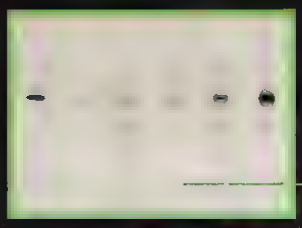

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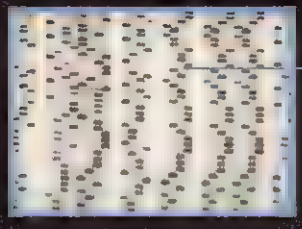

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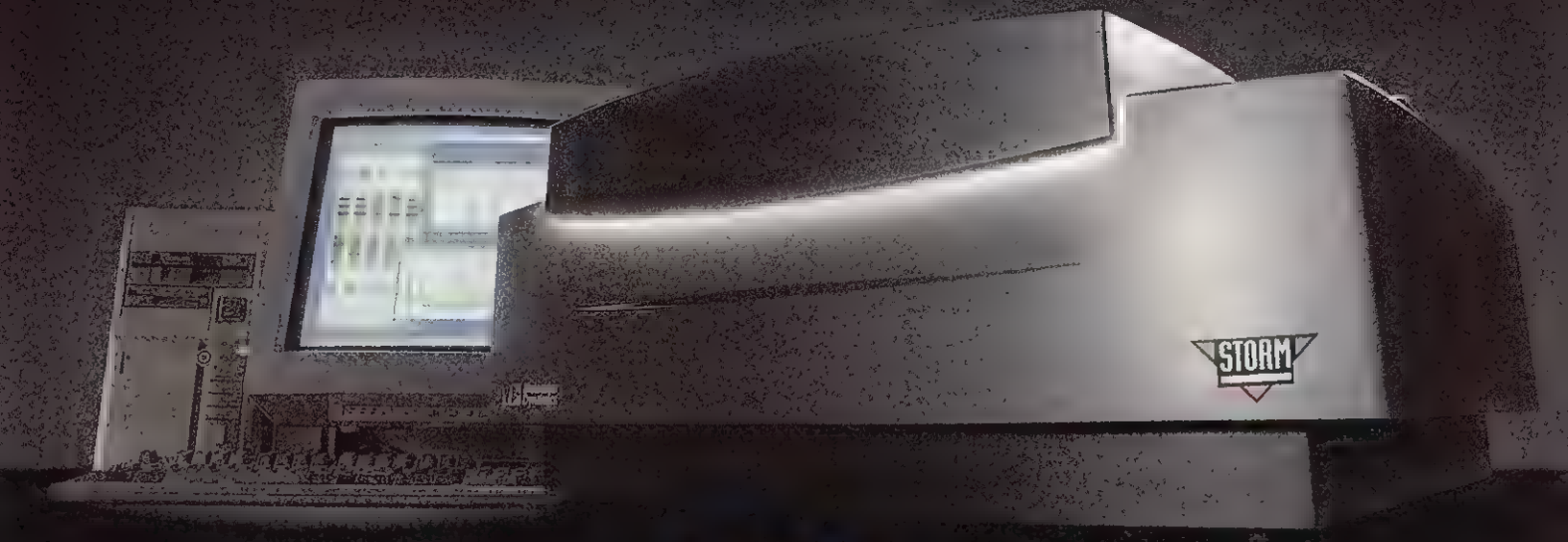

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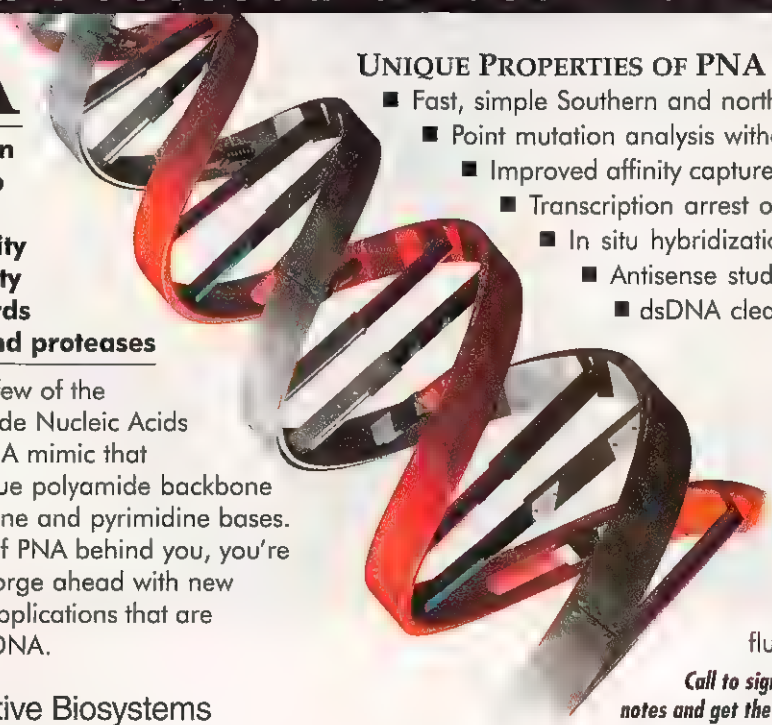

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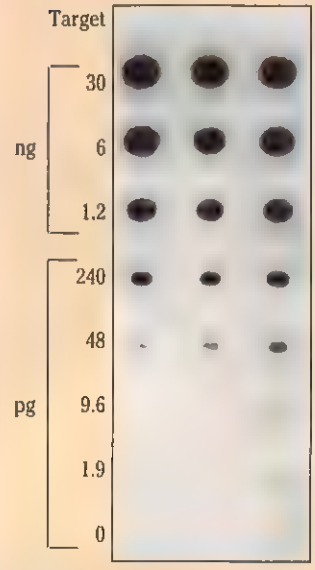

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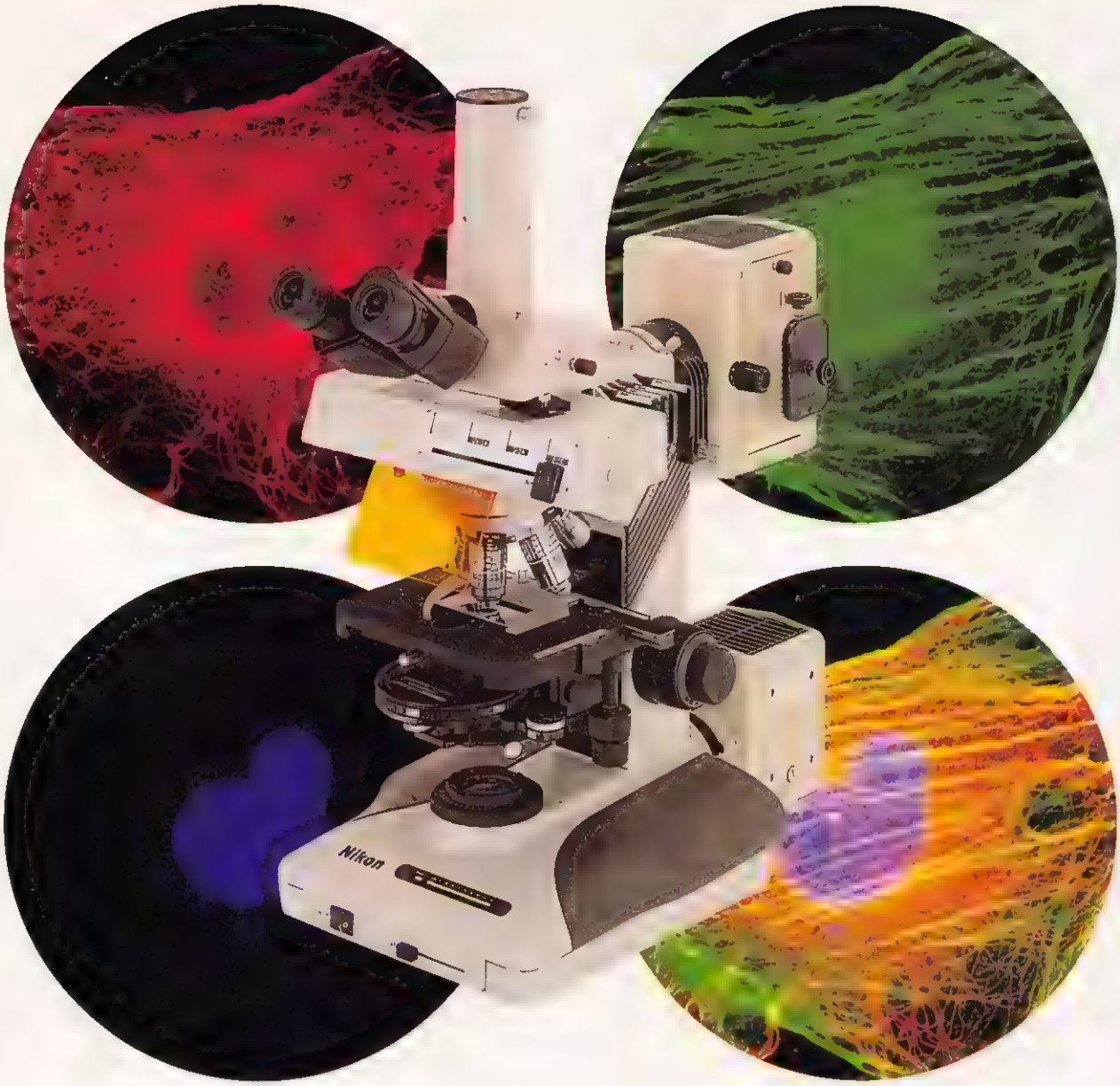

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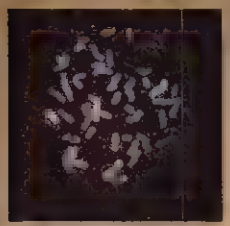

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Challenging an Ice-Core Paleothermometer

Doug MacAyeal

The temperature-depth profile near Earth's surface has long been recognized as an inventory of environmental history. In 1864 for example, Lord Kelvin used such a profile to argue that the age of Earth's crust since a presumed molten "birth" was inconsistent with prevailing notions of geologic time (*1*). Kelvin's age estimate turned out to be low because it did not account for radioactive heating within rocks and mantle connection which were discovered after the time of Kelvin's analysis. Nevertheless, an important lesson is appreciated from Kelvin's work (*2*): Environmental history (such as annual average surface air temperature) produces a unique effect on near-surface Earth temperatures.

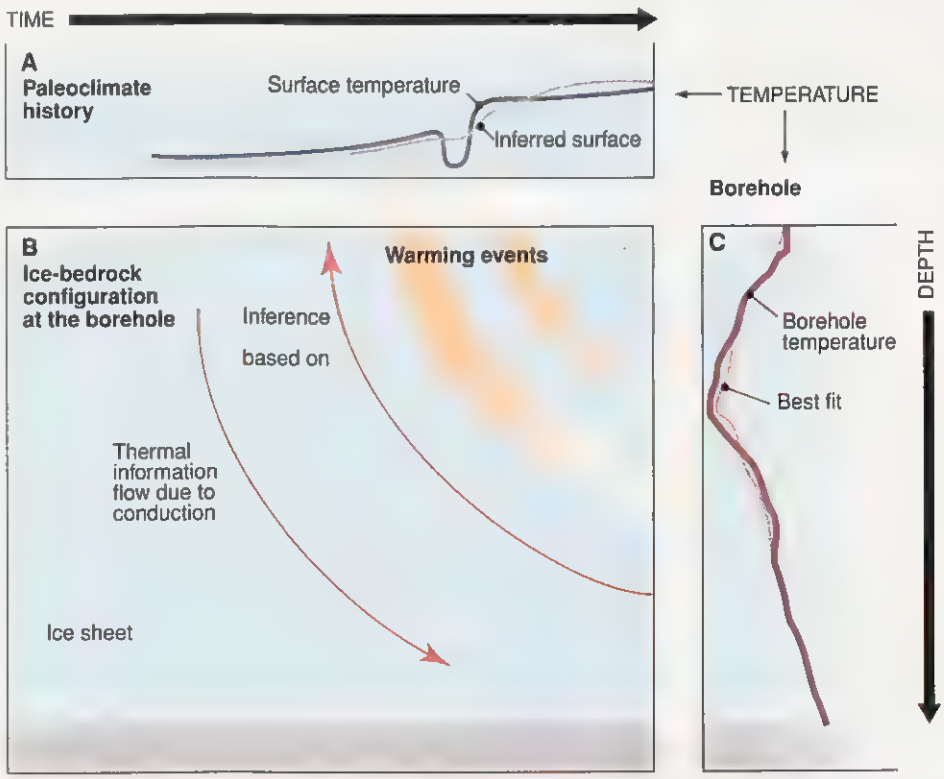
A fitting demonstration of Kelvin's lesson appears on page 455 of this issue, where Cuffey *et al.* report an analysis of temperature in the 3044-m-deep borehole at the GISP2 Greenland ice-core site (*3*). The first aim of their analysis is to challenge the fidelity of the surface-temperature history deduced previously from the ice-core oxygen-isotope stratigraphy of the GISP2 ice core. This oxygen-isotope derived history and a companion history derived by similar means from the GRIP ice-core site are very much on the minds of paleoclimatologists. They reveal an enigmatic climate-change pattern noted for its severity and abruptness (*4*). The challenge Cuffey *et al.* mount is fair because oxygen-isotope variation of ice is not strictly a function of local surface temperature alone, but is rather a compound function of the hydrologic process which delivers snow to the ice sheet. The second aim, pending the outcome of the first, is to calibrate the oxygen-isotope paleothermometer of the GISP2 ice core; that is, to quantify the relation between oxygen-isotope ratio shifts in the ice and temperature shifts at the time the ice was deposited.

The essence of the analysis of Cuffey *et al.* is a solution of the borehole-temperature paleothermometry problem for the GISP2 ice-core site. This problem is solved by estimating a 40,000-year surface-temperature history that successfully predicts the present-day temperature profile measured in the borehole. A solution to this problem is not easy to produce, nor is it easy to trust. The author of a recently published text on geophysical inverse theory refers to solutions of the borehole-temperature paleothermometry problem as "worthless for practical purposes" (*5*, p. 87).

The cause for this disclaimer is easily appreciated even without knowing much about the physics of heat flow in ice sheets or the mathematics of inverse methods. A surface temperature change penetrates an ice sheet by the combined effects of heat conduction and continual downward ice movement associated with snow accumulation (see figure). In the absence of heat conduction, downward movement would create an ideal one-to-one relation between borehole temperature at a given depth and surface temperature at a corresponding time.

Solution of the borehole-temperature paleothermometry problem would be a snap (much like what we desire ice-core oxygen-isotope paleothermometry to be) if this ideal, chronologically ordered thermal stratigraphy could be preserved. In actuality, the ideal thermal stratigraphy is not preserved because heat conduction erases temperature contrasts that are the signal of ancient surface-temperature change.

This "thermal memory" loss is a grave practical obstacle for the inference of a trustworthy temperature history. A flawed, untrustworthy solution (*6*) can display excessive, unrealistic oscillations that are constrained by nothing more than the minute, random errors in the borehole temperature measurements. Even adequate solutions have problems and can be trusted only to a limited extent because they can lack valid and interesting features, such as the Younger Dryas or Dansgaard-Oeschger events, that are too short-lived or too an-



A deeper look. The problem solved by Cuffey *et al.* (*3*) is illustrated by **B** (the ice configuration at the borehole site over time) showing how thermal information propagates through the ice sheet by downward ice movement and heat conduction. Surface temperature perturbations, such as the warming events shown **A**, penetrate the ice sheet and move down and to the right through the box with the passage of time. At the present day (right edge of **A**), the ice-sheet temperature is measured, yielding the profile shown in **C**. A backward-time calculation, or a series of trial forward-time calculations, are used to infer a surface temperature history that best predicts the borehole temperature data. The problem is difficult because heat conduction limits thermal memory. The result of this obstacle is that the inferred surface temperature history is not unique. Short-lived or ancient surface temperature events do not have sufficient influence on present-day borehole temperature to be resolved. Cuffey *et al.* overcome the nonuniqueness difficulty by selecting the history that most closely resembles the low-pass filtered (broad time-scale smoothed) ice-core oxygen-isotope chronology.

The author is in the Department of Geophysical Sciences, University of Chicago, Chicago, IL 60637, USA. E-mail: drm7@midway.uchicago.edu

cient to be preserved in the borehole temperature profile.

Practitioners of borehole-temperature paleothermometry cope with the obstacle of limited thermal memory by striking a balance between opposing goals. One goal is fidelity between borehole-temperature observations and the predicted borehole temperature profile based on the inferred surface temperature history. The other goal is simplicity of the inferred surface-temperature history, namely, sufficient simplicity to suppress the wild oscillations that would otherwise be introduced by random error in temperature measurement. These two goals are opposing, because improvements in satisfying one goal (say, simplicity of the temperature history) will detract from the satisfaction of the other (fitting borehole-temperature data).

Cuffey *et al.* strike the balance between the opposing goals in a remarkably adept manner that reflects their aim to challenge the ice-core isotopic paleothermometer (*3*). For the goal of simplicity, Cuffey *et al.* demand that the inferred surface-temperature history be linearly related to only the broadest time-scale patterns of the oxygen-isotope record. For the goal of fidelity, they minimize a least-square measure of misfit between the observed borehole temperature log and the temperature profile predicted by the inferred surface-temperature history. (This prediction involves a sophisticated ice-sheet temperature model, which is one of the technical highlights of their analysis.) The adjustable parameters, which are tuned to achieve this minimization, are simply the slope and intercept of the linear relation defining the ice-core oxygen-isotope paleothermometer. As can be appreciated from figure 2 of the report by Cuffey *et al.*, the twin opposing goals are achieved, and the ice-core oxygen-isotope paleothermometer is both confirmed and calibrated.

This success is qualified, however, by its applicability to only the broadest time-scale feature of the surface-temperature history of the GISP2 site, that is, the glacial to interglacial warm-up. Other interesting climate features implied by the ice-core isotopic record, such as the 10,000-year-old Younger Dryas event or the presence or lack of the controversial Eem/Sangamon cold spells, may never be confirmed. These ancient and short-lived features fall below the resolution threshold of borehole-temperature paleothermometry (*7*).

For scientists who generate and interpret ice-core paleoclimate data, the success of the analysis by Cuffey *et al.* is satisfying because it culminates several decades of effort to use borehole-temperature paleothermometry as a check on other techniques for deriving environmental histories. Of more general interest, their work implies that past estimates of the glacial to interglacial warm-up derived from the ice-core isotope records have been too conservative (that is, were 8°C when they should have been 16°C) (*8*). This deduction is in accord with similar conclusions drawn recently about glacial-period temperatures in the tropics (*9*) and confirms that polar amplification of climate change (for instance, the poles respond with greater amplitude than the tropics) is a central characteristic of Earth's climate.

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8. This conclusion, that the glacial to interglacial warm-up was much greater than previous interpretations of Greenland ice-core isotopic records indicated, was also achieved in a similar analysis by Cuffey's predecessor at the Geophysics Program of the University of Washington, J. Firestone. Firestone's study involved data from a Greenland ice-core site that was not as well suited for borehole-temperature paleothermometry as the GISP2 site. See (*7*).
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Life With 482 Genes

André Goffeau

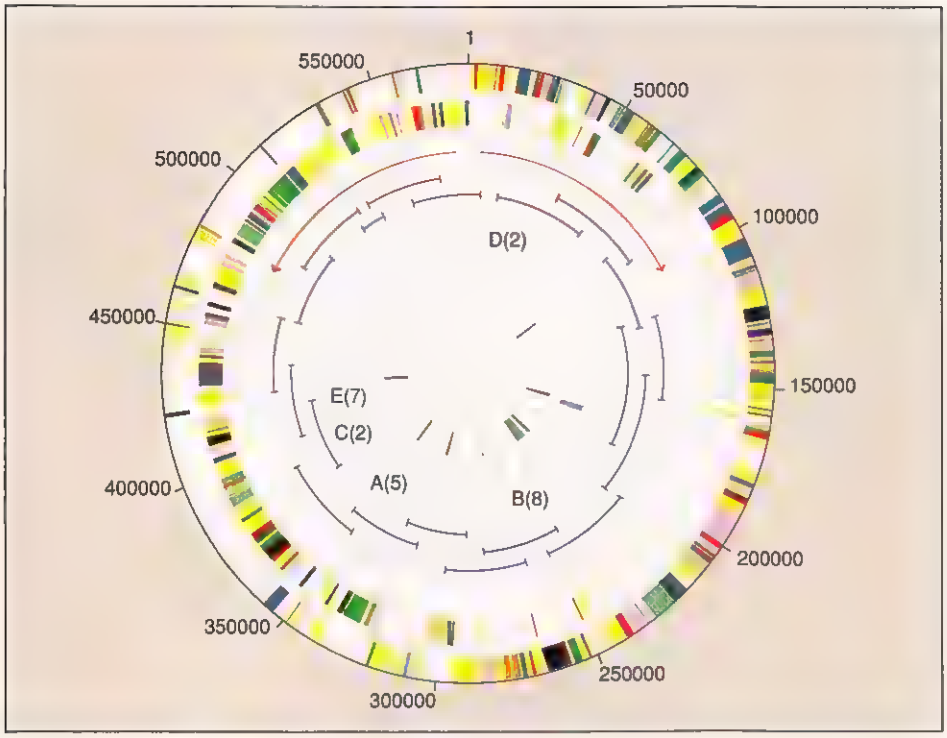
For many years we believed that the first genome to be sequenced entirely would be that of the bacterium *Escherichia coli*, estimated to be 4720 kilobases long (*1*). More recently, the prospect of completing the much longer genome of the yeast *Saccharomyces cerevisiae* (12,500 kilobases) before the end of 1995 has been entertained (*2*). But to everyone's surprise, an outsider won the race for the first complete genome sequence—that of the bacterium *Haemophilus influenzae*, a 1830-kb sequence, recently reported by a team from the Institute for Genomic Research (TIGR) headed by Craig Venter (*3*). This "premiere" was performed on a very small bacterial genome, and not on the long mammalian fragment on which one might have expected TIGR to have concentrated its powerful resources. Now, Venter and his colleagues have focused on an even smaller genome, that of *Mycoplasma genitalium*, the complete sequence of which is reported in the paper by Fraser *et al.* in this issue of *Science* (*4*). This parasite (but not necessarily a pathogen) of human genital and respiratory tracts has a genome of only 580-kb long.

Sequencing of the *M. genitalium* genome was the product of a collaboration among three teams. Foremost among these is TIGR, a nonprofit institute with large-scale, high-throughput DNA sequencing facilities that has given the rights to commercialize its findings to an allied company, Human Genome Sciences, Inc. TIGR and its director Craig Venter are known mainly for their mass production of sequence tags for human genes (ESTs) expressed in different tissues (*5*). The second team is led by Hamilton Smith from the Johns Hopkins University School of Medicine, who is best known for his pioneering work on restriction enzymes in bacteria, work that opened the field of molecular genetics and for which he won the Nobel Prize in 1978. The third team is that of Clyde Hutchison from the University of North Carolina, who is an internationally recognized expert in the study of *Mycoplasma* species.

One of the most impressive features of the sequencing effort for the *M. genitalium* genome is its efficiency, a testament to the power of the TIGR sequencing and informatics facilities. The first DNA extraction from *M. genitalium* was carried out in early January 1995, and the manuscript was submitted on 11 August 1995.

On the technical side, the most spectacular aspect of the work is the application of random ("shotgun") sequencing and assembly of the 8650 needed sequencing reactions in a single contig (that is, a collection of overlapping clones that collectively cover the target region). This accomplishment was made possible by the development of a series of highly performing informatics tools already tested during the sequencing of the *H. influenzae* genome. On the scientific front, the originality of the work stems from the fact that the *M. genitalium* genome has one of the smallest known genomes of any free-living organism. It is therefore reasonable to assume that its genome sequence reveals the near-

The author is at the Université Catholique de Louvain, Unité de Biochimie Physiologique, Place Croix du Sud, 2-20, B 1348 Louvain-la-Neuve, Belgium.



A circular representation of the *M. genitalium* chromosome. Outer concentric circle: Coding regions on the plus strand for which a gene was identified. Second concentric circle: Coding regions on the minus strand for which a gene was identified. Third concentric circle: The direction of transcription on each strand of the chromosome starting at the putative origin of replication. Fourth concentric circle: Cosmid and lambda clones (blue). Fifth concentric circle: The locations of the single ribosomal operon (blue) and the 33 tRNAs. The clusters of tRNAs (trnA, trnB, trnC, trnD, and trnE) are indicated by the letters A through E with the number of tRNAs in each cluster listed in parentheses. Sixth concentric circle: Location of the MgPa operon (green) and MgPa repeat fragments (brown). Figure generated with TIGR Genome Display Tool (J. Slagel, unpublished data). [Figure from C. Fraser *et al.*, unpublished data]

minimal set of genes necessary for independent life. Whether this means that each of the predicted coding regions is essential for growth remains to be determined experimentally. Nevertheless, it is likely that the minimal translation machinery requires nearly 90 different proteins to proceed, whereas the complete DNA replication process requires only about 30 proteins. Many other observations can be made on minimalist metabolic or physiological pathways. It is surprising, for example, that this bacterium, which contains only one type of membrane, has devoted 140 (30%) of its 482 genes to encode membrane-inserted proteins. Also unexpected is the observation that up to 4.5% of the genome might be used for evasion of the mammalian host immune response. The scope of our present ignorance is measured by the fact that 117 *M. genitalium* protein sequences (22%) do not match protein sequences from any other organism.

From an evolutionary perspective, sequencing of the *M. genitalium* genome represents the first complete molecular definition of minimal life and will likely become a cornerstone for future comparisons of the genome contents from many species. An example of such an approach is given by the comparison of the gene content of *H. influenzae* and *M. genitalium* sorted by functional category; for instance, *H. influenzae* devotes more than 10 times as many genes to regulatory functions than *M. genitalium* (64 genes compared with 5).

How rapidly will the new information be exploited at the biochemical level? Two facts might hinder such progress, which in principle could result in a definition of the minimal biochemical mechanisms and pathways required for life. One limiting factor might be the relatively small size of the scientific community actively working on molecular aspects of this bacteria (probably a few dozen compared to the thousands of molecular biologists studying yeast or *Escherichia coli*). Another difficulty is that the tools of classical or molecular genetics cannot be applied; no auxotrophic or other mutants are presently available for this parasite, which cannot grow on synthetic media. For further studies to take place, each of the *M. genitalium* genes should be overexpressed in a heterologous host—a procedure that remains cumbersome.

Another question concerns the true error rate of the sequence. The authors admit that about 1% of the total sequence was determined from only one DNA strand. This contrasts with the requirement for 100% coverage of both strands in other systematic sequencing projects, such as those of yeast or *Bacillus subtilis* genomes. Whether full coverage on the second strand is always necessary when the sequence on the opposite strand is known is indeed open to discussion. The authors estimate, rather intuitively, that their error rate is less than 1 in 10,000 bases. In some circles it is believed that this estimate, which corresponds to the highest accepted standards, should have been assessed by independent quality control on the basis of blind resequencing. The debate as to the measurement of exact error frequency should, however, in no way overeshadow this remarkable achievement, which will remain a landmark in contemporary biology.

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Cosmogenic Ages for Earthquake Recurrence Intervals and Debris Flow Fan Deposition, Owens Valley, California

Paul R. Bierman,* Alan R. Gillespie, Marc W. Caffee

Model exposure ages (beryllium-10, aluminum-26) of boulders on an offset debris flow fan yield an earthquake recurrence interval between 5800 and 8000 ^{10}Be:^{26}Al years for a strand of the Owens Valley fault in California, which last ruptured in an earthquake of moment magnitude >7.5 in 1872. Cosmogenic age estimates for this and several nearby fan surfaces flanking the eastern Sierra Nevada are consistent with stratigraphic relations and suggest that these surfaces were abandoned after 1000, 8000, and 21,000 ^{10}Be:^{26}Al years ago. The wide scatter and nonconcordance of ^{10}Be:^{26}Al ages on an older fan surface suggest that boulder erosion and lowering of the fan surface there have influenced apparent exposure ages.

The east front of the Sierra Nevada rises about 3000 m from the floor of Owens Valley (Fig. 1). Uplift, fluvial down-cutting, and repeated glaciation during the Pleistocene have etched deep canyons into the range front. At the mouths of these canyons, extensive alluvial fans merge into a gently sloping surface bordering the range and extending to the valley center, a distance of up to 10 km. These fans are bouldery and are formed in large part by debris flows (*1*). Near Lone Pine (36° 36′N, 118° 05′W), boulders are commonly granitic, equant, and 2 to 4 m in diameter; the largest exceed 10 m. Fan-building episodes and increased frequency of debris flows have been correlated with deglaciation in the Sierra Nevada (*2*) primarily on the basis of terraces mapped inside equivalently weathered moraines and on arguments regarding the availability of sediment from glacially overdeepened source basins. In this report, we present cosmogenic age estimates for the timing of Sierra Nevada fan aggradation, and we use these estimates to calculate average recurrence intervals for movement on the Lone Pine fault.

Along Lone Pine Creek (Fig. 1), cross-cutting relations and relative weathering criteria distinguish geomorphic surfaces of at least three distinct ages (*3*). The oldest surface, Qg1, is smooth and underlain by heavily weathered granitic boulders; deposits of equivalent weathering intensity appear to underlie much of the bajada. Qg1 likely represents more than one episode of deposition, but we cannot distinguish them reliably at Lone Pine with relative weathering criteria. Boulders on the Qg1 surface are rare, heavily weathered, and primarily aplitic. Fan surface Qg3 has abundant, moderately weathered granitic boulders and distinct boulder levees. Qg4, a terrace of Lone Pine Creek, is the youngest surface on which we collected samples for dating. It is inset as much as 20 m into the Qg3 surface and is covered by large, unweathered granitic boulders. Although the stratigraphic relations among these three surfaces are certain, their relation to a fourth and faulted fan surface 8 km down Lone Pine Creek is ambiguous (Fig. 2). Relative weathering data indicate that the faulted fan is likely younger than the Qg1 and Qg3 fan surfaces (*4*).

The faulted fan has been beheaded by stream capture and is displaced vertically more than 6 m by the Lone Pine fault (LPF), a strand of the Owens Valley fault (OVF), which runs along the valley bottom for more than 100 km and in several places cuts the Los Angeles aqueduct (Fig. 2). In 1872, the OVF ruptured for a distance of 100 ± 10 km, generating an earthquake of estimated moment magnitude 7.5 to 7.7 (*5*). During this event, right-lateral, oblique slip on the LPF broke the fan surface. Observations from trenches (*4*) suggest that three events are recorded by colluvial wedges in the adjacent graben; this observation is supported by scarp morphology as well as desert-varnish rings (*4*) and weathering

Table 1. Isotopic data, Lone Pine Creek debris fans.

Sample	^{10}Be (10^5 atoms g^{-1})*	^{26}Al (10^5 atoms g^{-1})*	^{10}Be model age† (10^3 years)	^{26}Al model age† (10^3 years)
		Surface Qg1		
LPF-2	22.64 ± 0.75	111.23 ± 9.94	97.3 ± 21.2	77.7 ± 19.2
LPF-20	27.45 ± 1.07	90.04 ± 5.10	125.7 ± 28.1	67.8 ± 15.7
LPF-1	10.45 ± 0.41	45.73 ± 2.85	45.5 ± 9.6	32.7 ± 7.2
AHI-1	17.28 ± 0.62	95.70 ± 5.48	88.8 ± 19.2	79.7 ± 18.8
AHI-14	25.11 ± 0.74	75.84 ± 4.12	127.9 ± 28.4	63.7 ± 14.6
AHI-16	20.13 ± 0.62	101.54 ± 7.01	103.9 ± 22.7	84.4 ± 20.4
Average			98.2 ± 30.1 (12.3)	67.3 ± 18.8 (7.7)
		Surface Qg3		
LPF-16	9.46 ± 0.38	54.83 ± 5.62	37.2 ± 7.8	35.3 ± 8.4
LPF-17	5.34 ± 0.25	31.47 ± 2.26	21.1 ± 4.4	20.4 ± 4.5
LPF-18	5.34 ± 0.25	35.34 ± 4.11	21.1 ± 4.4	22.9 ± 5.5
LPF-19	5.04 ± 0.26	32.92 ± 2.69	22.8 ± 4.8	24.5 ± 5.5
LPF-8	4.68 ± 0.21	27.51 ± 2.42	21.2 ± 4.4	20.5 ± 4.6
LPF-9	5.56 ± 0.26	27.21 ± 3.00	25.2 ± 5.3	20.3 ± 4.8
AHI-5	5.54 ± 0.45	28.33 ± 3.15	29.1 ± 6.4	24.3 ± 5.8
Average			25.4 ± 6.0 (2.3)	24.0 ± 5.3 (2.0)
		Surface Qg4		
LPF-3	0.25 ± 0.07	1.82 ± 0.48	1.1 ± 0.4	1.3 ± 0.4
LPF-5	0.23 ± 0.16	3.20 ± 0.48	1.0 ± 0.7	2.3 ± 0.6
LPF-6	0.22 ± 0.09	4.59 ± 0.74	1.0 ± 0.4	3.3 ± 0.9
LPF-7	0.37 ± 0.24	2.72 ± 0.37	1.6 ± 1.1	2.0 ± 0.5
LPF-4	0.23 ± 0.29	1.14 ± 0.10	1.1 ± 1.3	0.8 ± 0.2
Average			1.2 ± 0.3 (0.1)	2.0 ± 1.0 (0.4)
		Faulted fan		
LPF-11	1.64 ± 0.19	11.74 ± 1.26	11.5 ± 2.7	13.5 ± 3.1
LPF-12	1.16 ± 0.10	6.78 ± 0.85	8.2 ± 1.8	7.8 ± 1.9
LPF-13	1.52 ± 0.14	9.44 ± 1.57	10.7 ± 2.4	10.9 ± 2.9
LPF-14	2.54 ± 0.19	14.78 ± 2.15	17.8 ± 3.8	17.0 ± 4.3
LPF-15	1.41 ± 0.13	8.10 ± 1.21	9.9 ± 2.2	9.4 ± 2.4
Average			11.6 ± 3.7 (1.6)	11.7 ± 3.6 (1.6)

*Propagated uncertainties include blank and carrier, total Al (5%), and total Be (2%). †Assuming sea level, high altitude production rates of 6.03 (^{10}Be) and 36.8 (^{26}Al) atoms g^{-1} $year^{-1}$ (±20%). Altitude-latitude corrections are according to (*15*) assuming all production by nucleons. Values in parentheses for the average model ages are standard errors of the mean. Propagated uncertainties include stated uncertainty in isotopic abundance and 20% uncertainty in production rates.

P. R. Bierman, Department of Geology, University of Vermont, Burlington, VT 05405, USA
A. R. Gillespie, Department of Geological Sciences, University of Washington, Seattle, WA 98195, USA
M. W. Caffee, Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, Livermore, CA 94550, USA.

*To whom correspondence should be addressed.

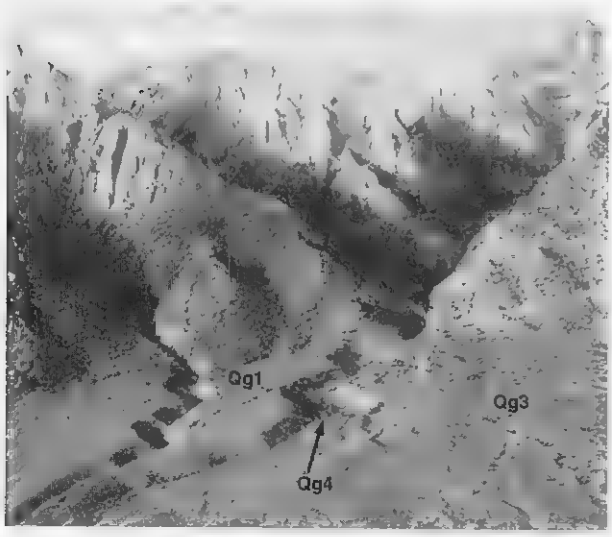
zones on a large (4-m) boulder (sample LPF-14) exposed in and above the fault scarp. The first faulting event recorded by sediments in the graben probably occurred before the faulted fan was beheaded by stream capture, which prevented further deposition (*4*).

Both the actual and average recurrence intervals of ground-rupturing earthquakes on the LPF had been difficult to determine because, until this study, neither the offset fan surface nor the faulting events had been dated directly (*6*). Previously, recurrence intervals (5000 to 10,500 radiocarbon years) were calculated by assigning the faulted fan a minimum, late glacial age of 10 ka (thousand years ago) and by assuming that the fan, because it does not preserve a shoreline of Pleistocene Lake Owens, was younger than radiocarbon-dated shoreline tufa (21,000 ± 1300 radiocarbon years, sample USGS-609) (*4*). To estimate the age of the faulted fan surface and to investigate the utility of in situ–produced cosmogenic isotopes for dating debris flow fan surfaces, we collected and analyzed 23 samples from granitic boulders on four fan surfaces near Lone Pine (*7*). To calculate model ages from measured isotope abundances, we used currently accepted isotope production rates, which may be 10 to 20% too high (*8–10*). Because production rates of ^{10}Be and ^{26}Al remain uncertain, we interpret isotope abundances as model ages propagating a production rate uncertainty of ±20% (*10*).

Our isotopic data provide a direct estimate of the timing of boulder deposition and abandonment of the faulted fan and allow calculation of average recurrence intervals for ground rupture on the LPF (Table 1). Model ^{10}Be:^{26}Al ages (assuming no isotopic inheritance and no boulder erosion) for five boulders on the faulted fan range from 8.0 to 17.4 ka with a mean exposure age of 11.7 ka. The ^{10}Be and ^{26}Al model ages are well correlated (Fig. 3), suggesting that the variability in ages is not due to analytic uncertainties, but rather mainly reflects differing amounts of isotope inheritance from predeposition exposure to cosmic rays, effects of exposure geometry, boulder erosion, and time-transgressive deposition on the fan surface. Although we cannot chose confidently one explanation over another, we favor the latter explanation for three reasons: (i) The Qg4 data indicate isotope inheritance is minimal, (ii) the sampled boulders are not heavily weathered (*4*), and (iii) the boulder with the lowest average ^{10}Be:^{26}Al exposure age (LPF-12; 8.0 ka) is located at the margin of the youngest abandoned channel (LPC-2), which was probably active after the first faulting event (*4*), whereas desert-varnish rings show that the boulder with the oldest ^{10}Be:^{26}Al average exposure age (LPF-14; 17.4 ka) is located near an older abandoned channel and records all three faulting events (*4*).

There are various ways to calculate a recurrence interval for ground rupture on the LPF assuming that faulting recurs at characteristic, regular intervals. At the simplest, if the fan surface age were taken to be the average age of the boulders on it (11.7 ka, $n = 5$) and the fan has been offset by three events (*4*), then the average recurrence interval would be 5850 years; however, the faulted fan is composed of debris flows emplaced over a period of time and therefore has no single age. Lubetkin and Clark (*4*) pointed out that three faulting events ruptured the southern, older portion of the fan near boulder LPF-14 (17.4 ka) from which we calculate an average recurrence interval between 5800 to 8700 years. To the north, the fault scarp offsets the last active channel of Lone Pine Creek on the faulted fan and only two events are preserved there. LPF-12 (8.0 ka) was deposited or exposed on the margin of this channel (LPC-2) after the first faulting event but before the fan was abandoned and the second and third faulting events occurred (*4*), yielding an average recurrence interval between 4000 and 8000 years. Conservatively, the average earthquake recurrence interval on the Lone Pine fault is the outer limits, 4000 to 8700 ^{10}Be:^{26}Al years; however, 5800 to 8000 years is the range of recurrence intervals consistent with the dating at both sites.

The recurrence intervals we have calculated are similar to those previously calculated (*4*); however, our calculations are more robust because we estimated the age of the fan surface directly. Our dating also suggests that deposition on the faulted fan continued through the late Pleistocene before early Holocene stream capture and abandonment of the faulted fan <8000 ^{10}Be:^{26}Al years ago (LPF-12). This finding is consistent with radiocarbon dates on charcoal (610 ± 70 ^{14}C years ago, sample QL-4361; 4030 ± 60 ^{14}C years ago, sample TO-1666) which indicate that Holocene fluvial activity and fine-grained debris flows have deposited material on the Qg5 or modern fan (Fig. 2). Cosmogenic dating of the Qg4 surface and radiocarbon dating of the Qg5 fan suggest that debris flow activity and fan deposition continued through the Holocene and that the debris flow that



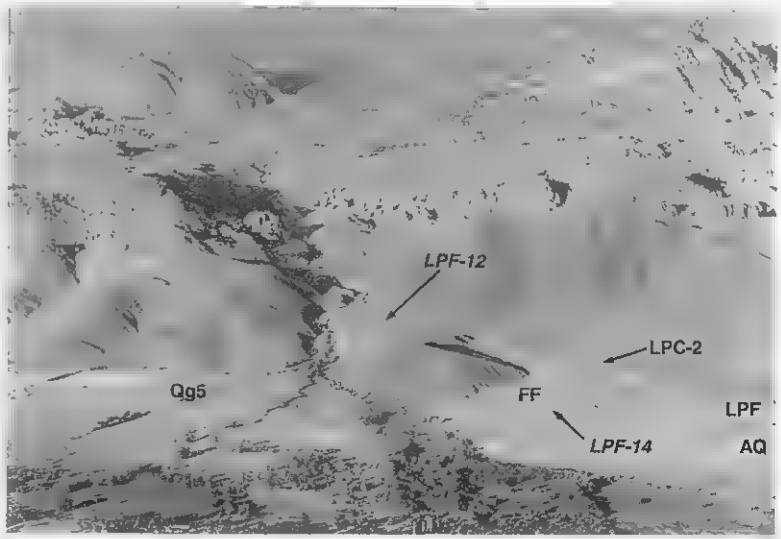


Fig. 1 (left). Oblique aerial photo looking west toward the east front of the Sierra Nevada and over fan surfaces Qg1, Qg3, and Qg4 of Lone Pine Creek. Lone Pine Creek is inset into surface Qg4. The road paralleling Lone Pine Creek indicates scale. **Fig. 2 (right).** Oblique aerial photo of Alabama Hills west of Lone Pine, California. A faulted and beheaded late Pleistocene fan is on the right (FF). On the left is the active, Holocene fan (Qg5). Sample sites LPF-12 and LPF-14 and the abandoned channel, LPC-2, are identified with arrows. LPF, Lone Pine fault scarp; AQ, Los Angeles aqueduct. Houses and roads indicate scale. View to west.

blocked the Los Angeles aqueduct at Olancha during the summer of 1990 is not an isolated geologic hazard in Owens Valley. However, the relatively small volume of unweathered boulders on Owens Valley fans and the deep incision of Lone Pine Creek suggest that the rate of Holocene debris flow deposition is lower than during the Pleistocene.

The three other fan surfaces have consistent relative and cosmogenic ages. Using the five samples from unweathered debris-flow boulders on the youngest, inset Qg4 surface, we have demonstrated that cosmogenic isotope abundances can be measured successfully in late Holocene samples. Our results indicate that deposition on the Qg4 surface ceased about 1000 years ago. The discordance between some Qg4 ^{10}Be and ^{26}Al ages may reflect inheritance from prior exposure, preferential radiogenic or muonogenic production of ^{26}Al, or errors in blank correction for these very low-level samples. The low isotopic abundances measured in samples from the Qg4 surface suggest that for boulders on debris flow fans in Owens Valley, isotopic inheritance from cosmic ray exposure before deposition on the fan is probably minimal (≤2.0 ka). This lack of significant prior exposure contrasts with apparent inheritance for clasts sampled from predominately fluvial fans in Death Valley, 100 km east (*11*).

The extensive Qg3 surface (mean ^{10}Be: ^{26}Al age, 25 ka) represents the end (by 21 ka) of an earlier depositional period and provides a maximum limiting age for the deep fan-head incision of Lone Pine Creek, after which boulders could no longer be deposited on the Qg3 surface (Fig. 1). The clustering of six of the seven ages suggests that erosion by fire spalling has been minimal or similar among boulders (*12*). The single boulder with the higher exposure age (LPF-16; 36 ka) lies farther from the fan axis and closer to the mountain front than all other samples and may represent either an earlier episode of fan deposition or an isolated rockfall from the Sierran escarpment. Isotopic measurements on the Qg3 surface suggest that late Pleistocene, Sierra Nevada debris-fan surfaces likely preserve a consistent age signal and that a significant source of bouldery debris (extensive glaciation?) was available to supply material for deposition on the Lone Pine fans between 21 and 26 ka.

The magnitude and variety of model exposure ages calculated for boulders on the Qg1 surface are consistent with the oldest relative age of this surface, the degree of postdepositional surface lowering, and the magnitude of boulder weathering. The discordance of Al and Be ages (age ^{10}Be > age ^{26}Al) is consistent with a scenario in which sampled boulders were exposed to cosmic radiation, then buried by debris flows and re-exhumed by fan surface lowering (Fig. 3). During burial, more of the relatively short-lived ^{26}Al would decay than would the longer lived ^{10}Be. The large range of model exposure ages on this surface probably represents differing times of boulder burial and exhumation, differing rates of boulder erosion after exhumation, and perhaps the time-transgressive nature of fan deposition. The Qg1 boulder with the lowest isotopic abundance (LPF-1) is coarse grained, heavily weathered, and stands <1 m above the fan surface. The remaining boulders plot on a line trending away from Be and Al correlation, suggesting differing exposure and burial histories (Fig. 3). Cosmogenic data can be used to suggest that deposition on Qg1 began before 128 ka (AHI-14), presuming none of the sampled boulders inherited ^{10}Be or ^{26}Al from cosmic ray exposure before deposition.

Current uncertainties in cosmogenic isotope production rates prevent meaningful comparison of our estimates for the timing of fan surface abandonment with global climate records such as δ^{18}O measured in ice and deep-sea sediments. The paucity of ^{10}Be and ^{26}Al measurements on samples from Sierra Nevada moraines precludes us from understanding the temporal relation between moraine and fan deposition. Taking existing data at face value, it appears that the cessation of fan deposition lags moraine deposition consistent with field stratigraphic evidence. Earlier ^{10}Be and ^{26}Al dates from latest Pleistocene moraines at Pine Creek (*11*), 120 km north of Lone Pine (13.8, 13.9, and 17.5 ka), suggest that these moraines predate the abandonment of the Lone Pine faulted fan and postdate abandonment of the Qg3 surface. Because boulders on the Qg1 fan surface appear to have complex burial and exposure histories, correlation with older Pine Creek moraine samples (95 and 115 ka) is not meaningful. Lastly, our model ages for fan aggradation can be compared with the only extensive cosmogenic data set published so far for Sierra Nevada moraines (*13*). From revised ^{36}Cl production rates, exposure ages of boulders from three nested moraines at Bloody Canyon, about 200 km north of Lone Pine, average 16.0, 17.3, and 36.7 ka (*14*). The two younger moraines predate abandonment of the faulted fan and postdate deposition on the Qg3 surface. The oldest moraine appears to predate abandonment of the Qg3 surface.

We have demonstrated that Sierra Nevada alluvial fans preserve a datable record of fan surface activity and abandonment. Better production rate estimates and more cosmogenic measurements of samples from moraine and fan surfaces may reveal the temporal relation between fan and moraine deposition and let us fully exploit these terrestrial archives of climate change.

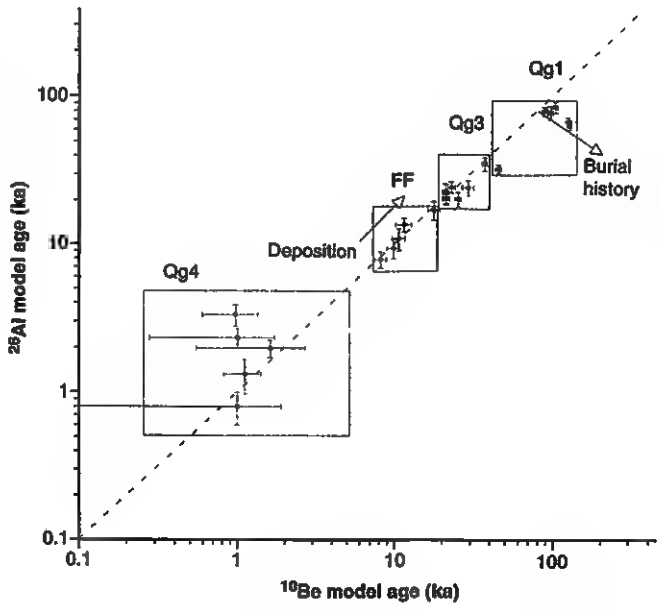


Fig. 3. Scatter plot of ^{10}Be and ^{26}Al ages. Samples from each fan surface are boxed and identified. FF, faulted fan. Error bars (1σ) include propagated counting statistics, blank and carrier correction, and total Al and Be abundance, but no uncertainty in production rates. Arrows show the likely time-transgressive nature of boulder deposition on the FF surface and differing burial and exposure histories of boulders on the Qg1 surface.

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4. L. Lubetkin and M. Clark, *Geol. Soc. Am. Bull.* **100**, 755 (1988). The age of an alluvial fan surface is difficult to define because deposition on most fans takes place over an extended period of time. For example, an exposure in the head of the faulted fan in the Alabama Hills shows numerous strata, the lower of which are heavily weathered, indicating that initial deposition on the faulted fan probably occurred before the late Pleistocene. The distance to the fan from the range front (8 km) suggests that only the largest and least viscous debris flows could travel far enough to reach the faulted fan. Considering these observa-

tions, the faulted fan was most likely built by many episodes of deposition over thousands to tens of thousands of years. For the three youngest fans, we have used the youngest average ^{10}Be:^{26}Al age as a limit for fan surface abandonment, cognizant of the limitations imposed by small sample sizes (which causes overestimate of the limit), boulder erosion (causes underestimate), and the assumption of no isotope inheritance at deposition (causes overestimate). A minimum duration of fan deposition can be estimated from the distribution of boulder ages; however, such an estimate is a function of analytic precision, the age distribution of boulders cropping out on the fan surface, and the number of boulders sampled and analyzed. If more samples are collected from any single fan surface, the apparent duration of deposition should generally increase as the likelihood of sampling boulders from the tails of the age distribution increases.

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6. Thermoluminesence (TL)–derived ages at Lone Pine were not useful for determining recurrence intervals because the TL system appeared not to have been reset to zero during erosion and transport of material from the scarp to the adjacent graben. No organic material suitable for radiocarbon dating has been found in four trenches dug so far across the LPF and adjacent graben; it is likely that the highly oxidizing environment destroyed most organic material.
7. Quartz was separated [C. P. Kohl and K. Nishiizumi, *Geochim. Cosmochim. Acta* **56**, 3583 (1992)] and ^{10}Be and ^{26}Al were isolated by cation exchange after HF dissolution at the University of Vermont. We measured isotopic ratios at the Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory. Measured ratios, corrected for blanks and carrier, ranged from 15×10^{-15} to 1989×10^{-15} and 52×10^{-15} to 3509×10^{-15} for $^{10}Be/Be$ and $^{26}Al/Al$, respectively. Isotope production, as a function of altitude and latitude, is scaled for nucleon abundance (*15*); for samples reported here, production is 2.4 to 4.2 times that at sea level.
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10. New radiocarbon data from the Sierra Nevada suggest that ^{10}Be and ^{26}Al calibration sites (*9*) are about 20% older than originally believed (13,000 to 14,000 calendar years ago versus 11,000 calendar years ago); thus, ^{10}Be and ^{26}Al production rates at sea level and high latitude may be up to 20% lower than the previously accepted values of 6.03 and 36.8 atoms g^{-1} $year^{-1}$, respectively (D. H. Clark *et al.*, *Geol. Soc. Am. Abstr. Programs* **26**, A447 (1994); D. Clark *et al.*, *Quat. Res.*, in press). This finding is supported by ^{10}Be and ^{26}Al data from the Laurentide terminal moraine in New Jersey (*8*). If production rates are revised downward, our model ages will increase by similar percentages. In addition to uncertainties in average production rates, changes in geomagnetic field strength have probably caused instantaneous production rates at Sierra Nevada altitudes and latitudes to vary over the duration of boulder exposure on the fan surfaces. Because production-rate calibration was performed on late Pleistocene surfaces (*9*), exposure ages could be overestimated by 10 to 15% for samples from the Qg1 surface, first exposed during a period of relatively high isotope production, and underestimated by a similar magnitude for samples from the Qg4 surface, which were exposed in part during a period of high field strength and consequently low production rate. We interpret isotope abundances as ages using 20% uncertainty in production rates propagating the following uncertainties: 10%, age of calibration surface (*9*); 6%, abundance variance in calibration samples (*9*); 10%, variation in magnetic field strength over time (*9*); 10%, altitude and latitude correction (*15*); 5%, muon contribution to production; and 5%, geomagnetic latitude of the sample over time. All in situ cosmogenic ages cited in this report are in ^{10}Be, ^{26}Al, and ^{36}Cl years which reflect an uncertain and nonlinear stretching of the calendar time scale.
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16. We thank J. Turner for assistance in sample preparation, C. Massey, D. Clark, K. Whipple, and K. Campbell for field assistance, and the staff at the Center for Accelerator Mass Spectrometry (Lawrence Livermore National Laboratory), in particular, J. Southon, for assistance in making the isotopic measurements. TL measurements made by G. Berger. Reviews by M. Clark and M. Pavich greatly strengthened this manuscript. Supported by NSF grant EAR 9004252 and 9396261, U.S. Geological Survey grant 14-08-001-G1783, and the University of Vermont (P.R.B.), and by NSF grant EAR 9004252 and the Geology program at the National Aeronautics and Space Administration (A.R.G.).

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Lithoautotrophic Microbial Ecosystems in Deep Basalt Aquifers

Todd O. Stevens* and James P. McKinley

Bacterial communities were detected in deep crystalline rock aquifers within the Columbia River Basalt Group (CRB). CRB ground waters contained up to 60 μM dissolved H_2 and autotrophic microorganisms outnumbered heterotrophs. Stable carbon isotope measurements implied that autotrophic methanogenesis dominated this ecosystem and was coupled to the depletion of dissolved inorganic carbon. In laboratory experiments, H_2, a potential energy source for bacteria, was produced by reactions between crushed basalt and anaerobic water. Microcosms containing only crushed basalt and ground water supported microbial growth. These results suggest that the CRB contains a lithoautotrophic microbial ecosystem that is independent of photosynthetic primary production.

The existence of microorganisms in the deep terrestrial subsurface has been noted for decades (*1*); viable microorganisms are present at depths as great as several thousand meters below the surface, in broadly variable physical and chemical settings (*2*). Nutrient flux at such depths is usually very low because of limitations of sediment chemistry and hydrology. The few measurements of in situ metabolic rates from these systems are the lowest recorded, which indicates that although microorganisms are active at such depths, they function in Earth's most oligotrophic environments (*3*). Most reported subsurface communities are ultimately, though indirectly, dependent on photosynthesis for energy; they either use remnant organic carbon deposited with sediments or use dissolved oxygen as a metabolic terminal electron acceptor. As nutrients are exhausted from sediments, the enclosed microbial population should become extinct. Here, we report evidence for an active, anaerobic subsurface lithoautotrophic microbial ecosystem (SLiME) within the CRB that appears to derive energy from geochemically produced hydrogen. SLiMEs should persist independently of photosynthetic products.

The CRB is a series of Miocene tholeiitic continental flood basalts that formed 6 to 17 million years ago and cover >163,000 km^2 (*4*). In our study area (Fig. 1), the CRB is 3 to 5 km thick. With increasing depth, the age of the water in confined aquifers between basalt flows increases (ages may exceed 35,000 years), as does the lateral distance to recharge. Shallow ground waters are low-sulfate, low-chloride bicarbonate solutions of moderate pH (generally 7.5 to 8.5), with calcium as the dominant cation. At depth, sodium and chloride predominate, and pH varies from 8 to 10.5 (*4*, *5*). Sulfate concentrations are below 0.5 mM even at depth, except in geographically restricted zones where sulfate concentrations may exceed 2.0 mM. The igneous rocks in the study area contained little organic carbon, yet we found relatively high populations of anaerobic microorganisms within aquifers hundreds of meters below any sedimentary interbeds (*6*).

To identify the electron acceptors and electron donors to which CRB communities are adapted (*7*), we investigated the metabolic capabilities of bacteria from eight aquifers. We measured the population sizes of bacteria capable of dissimilatory Fe(III) reduction (DIRB), sulfate reduction (SRB), methanogenesis (MB), fermentation (FB), or acetogenesis (AB). We also compared numbers of organisms that could grow on simple organic compounds (heterotrophs) with numbers of organisms that could grow with H_2 as the sole electron donor (autotrophs). The aquifers were sampled (*8*) through a series of preexisting wells (Fig. 1). The results of geochemical measurements (Table 1) were consistent with microbiological measurements (Table 2). DIRB were present only at low numbers, FB were com-

Pacific Northwest Laboratory, Post Office Box 999, Richland, WA 99352, USA. E-mail: to_stevens@pnl.gov or jp_mckinley@pnl.gov

*To whom correspondence should be addressed.

mon, and AB and MB were ubiquitous, although MB numbers were low in the high-sulfate ground waters. In contrast, SRB numbers were high primarily in the high-sulfate waters. In nearly every sample, autotrophic microorganisms outnumbered heterotrophic microorganisms by several orders of magnitude. Although H_2 is a common bacterial electron donor, this finding contrasts with observations of a variety of subsurface sediments and surface soils (9).

High concentrations of dissolved methane have been observed locally in the CRB (Fig. 2A) and natural gas was commercially exploited early in this century, but the origin of the gas is uncertain; stable isotope data suggest that it is largely of biogenic origin (*10*). Hydrogen concentrations are also relatively high (Fig. 2B), mostly three or more orders of magnitude above the range of 0.05 to ~10 nM that would be expected from microbial fermentation of organic matter (*11*, *12*). Thus, dissolved H_2 is widely present in the CRB in nonlimiting concentrations sufficient to promote microbial metabolism (*12*).

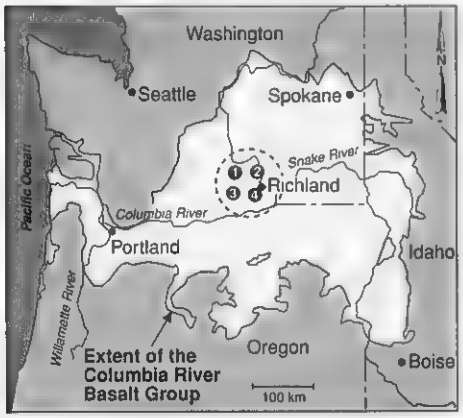

Fig. 1. Areal extent of the Columbia River Basalt Group and locations of the sampling wells used in this study. An extensive array of monitoring wells (within the dotted circle) was used for Figs. 2 and 3. Wells used for Tables 1 and 2 were located at numbered points (1, DB; 2, DC; 3, Pr; and 4, Jw).

Stable carbon isotope ratios of dissolved inorganic carbon (DIC) and methane suggest that the organisms in CRB ground waters are indeed active in situ. DIC is increasingly enriched in ^{13}C (*13*) at depths greater than ~200 m, consistent with the preferential removal of ^{12}C by methanogenic microorganisms (Fig. 3A). A smaller group of ground waters is depleted in ^{13}C and contains >0.5 mM dissolved sulfate; this observation suggests that sulfate-reducing bacteria oxidize biologically fixed carbon, which is relatively rich in ^{12}C. Thus, the electron acceptors available in the aquifer control the composition of microbial communities in the CRB.

During the production of methane, carbon is isotopically fractionated between methane and DIC. If the metabolism of H_2 and CO_2, as opposed to the metabolism of organic matter (acetate fermentation), were the primary process controlling methane formation, the effects of this process should be reflected both in concentrations of DIC and in the stable isotope compositions of that carbon. A characteristic depletion of $\Delta\delta^{13}C_{CO_2\text{-}CH_4} \approx -60$ parts per mil may be expected when bacteria form methane by reduction of CO_2 ($H_2 + CO_2 = CH_4$), as opposed to lesser fractionation ($\Delta\delta^{13}C_{CO_2\text{-}CH_4} \approx -20$ to -40 parts per mil) when bacteria grow on acetate ($C_2H_4O_2 = CH_4 + CO_2$), through which electron flow would occur during organic matter fermentation. To evaluate the hypothesis that CO_2 reduction was the dominant methanogenic pathway in CRB ground waters, we applied a fractionation model to existing data (*14*). Concentrations of DIC were assumed to decrease as a result of methanogenesis (Fig. 3B), and the concomitant expected change in $\delta^{13}C_{DIC}$ was calculated for comparison with measured $\delta^{13}C_{DIC}$. As shown in Fig. 3C, $\delta^{13}C_{DIC}$ values predicted by this method agreed with measured values. Calculations of expected $\delta^{13}C_{DIC}$ resulting from the acetate fermentation pathway ($C_2H_4O_2 = CH_4 + CO_2$) could not be adequately constrained.

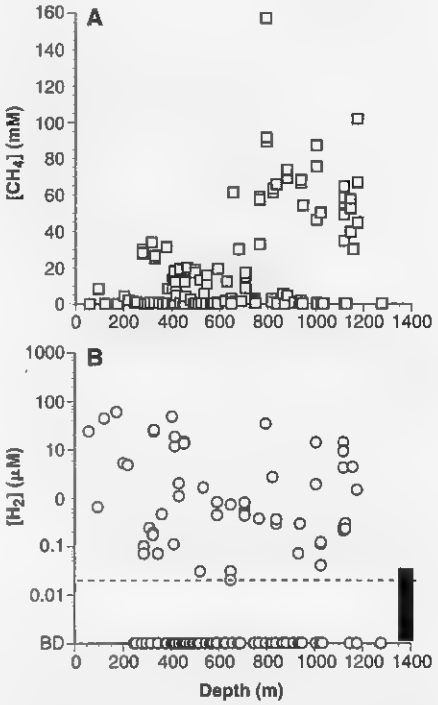

Fig. 2. (**A**) Dissolved methane and (**B**) hydrogen in CRB ground waters. Most data are from (*36*), with concentrations calculated after (*10*). These data were derived from an extensive system of monitoring wells located within the circled area in Fig. 1. In (B), the data are on a logarithmic scale (BD, below detection limits), the dashed line indicates the detection limit, and the vertical bar at the right represents the range of hydrogen concentrations expected during microbial oxidation of organic matter in sediments (*11*). Five different sampling methods were used to obtain these samples, including 26% of the total by artesian flow. There were no statistical differences between values obtained with different sampling methods, which suggests that sampling artifacts were minimal (*12*).

Table 1. Geochemical properties of ground-water samples from eight CRB aquifers. Well location codes are from Fig. 1; well numbers differentiate multiple wells at each locale. NA, data not available; <, below detection limit. Concentrations are millimolar, except as noted.

Analyte	Well number and solute values								Detection limit
	DC-06	DB-11	Jw-1	Jw-2	Jw-3	Pr-3	Pr-4	Pr-5	
Na	11.600	1.368	1.429	1.136	1.586	2.486	1.980	3.654	0.009
K	0.223	0.273	0.252	0.221	0.297	0.279	0.257	0.356	0.011
Ca	0.059	0.375	0.464	0.813	0.556	0.515	0.770	0.101	0.003
Mg	0.014	0.300	0.825	0.895	0.791	0.338	0.518	0.053	0.003
H_4SiO_4	1.789	0.958	1.022	0.956	1.086	0.799	0.769	1.020	0.003
F	1.905	0.040	0.025	0.029	0.024	0.040	0.027	0.076	0.001
Cl	4.568	0.120	0.167	0.174	0.179	0.281	0.261	0.240	0.002
NO_3	<	<	0.007	0.004	<	<	<	<	0.0002
SO_4	1.484	<	0.215	0.618	0.376	0.004	0.021	<	0.0005
Dissolved O_2	<	<	<	0.019	<	<	<	<	0.006
Sulfide (μM)	31.600	0.200	36.200	<	93.300	13.900	9.800	4.200	0.100
CH_4 (μM)	2	209	25	NA	16	481	135	185	1
pH	9.92	7.94	7.86	7.50	7.74	8.06	8.04	8.67	

Because DIC decreases with depth and acetate fermentation produces CO_2, an arbitrary sink for excess CO_2 was required in the calculations. The dependence of $\delta^{13}C_{DIC}$ on both addition and removal of DIC prevented a quantitative evaluation and argued against this mechanism as a control of $\delta^{13}C_{DIC}$. The success of the fractionation model lends further support to the hypothesis that abiotically produced H_2 supports microbial communities in the CRB. In the CRB, anaerobic H_2 oxidizers may be primary producers of organic carbon rather than facilitators of the catabolic terminal electron-accepting process, as they are in standard models of sediment metabolism (*15*).

We hypothesized that H_2O reduction to produce H_2, driven by iron in ferromagnesian silicates within the CRB, could serve as the abiotic energy source in this microbial ecosystem (*16*). We based this hypothesis on occurrences of free H_2 associated with ultramafic bodies in Earth's crust. H_2 has been observed at high concentrations (>10 volume % of exsolved gas) in nonvolcanic environments associated with serpentinized mafic and ultramafic rocks (*17, 18*). These occurrences apparently result from the weathering of Fe(II)-bearing silicates (such as olivine and pyroxene) at high rock:water ratios, where the dissolution of ferrous silicates and the precipitation of magnetite (Fe_3O_4) and other secondary phases drive the formation of H_2 (*19*). Weathering reactions may also be responsible for the alkalinity of ground waters observed in association with ultramafic bodies (*20*), and they can proceed and generate H_2 at ambient temperatures (*18, 21*). These proposed mechanisms are extrapolations from field observations and thermodynamic calculations; the precise reactions that are responsible for H_2 generation have not been demonstrated in controlled laboratory experiments. However, it is reasonable to expect that similar reactions would take place in CRB aquifers (*22*).

Ferrous silicates are not as abundant volumetrically in the CRB as in ultramafic rocks, but H_2 is also present at concentrations far below saturation in this system, which implies a much lower H_2 production rate. However, the abundance of ferrosilicate minerals does not necessarily limit the volume of product that can accumulate, because redox conditions during water-rock interaction are a function of the presence of ferrosilicates and dissolved oxygen, and not of mineral composition or volumetric abundance (*19*). The concentration of H_2 in CRB aquifers may be limited by the reacting surface area per unit volume of ground water and by the abundance of bacteria that make use of H_2.

In an attempt to produce direct evidence for H_2 production from basalt-water reactions, we conducted experiments with a variety of crushed rock samples. Steel is a common component of drill cuttings because of the abrasion of sampling and processing tools, and Fe(0) in steel may interact with water to produce H_2 (*23*). To rule out this source of hydrogen, we prepared steel-free crushed basalt samples from a well-characterized CRB outcrop (*24*) with the use of only stone and ceramic tools. Other rock samples were prepared in the same way. When basalt was added to pH-buffered water in sealed tubes under strictly anaerobic sterile conditions in the dark,

Table 2. Numbers of microorganisms in the eight CRB ground-water samples (om, organisms that grew on organic substrates; H_2, organisms that grew on $H_2 + CO_2$ only). The data are logarithms of numbers of organisms per milliliter belonging to various functional groups by enrichment series. The maximum detection limit was 10^4 organisms (+, growth in enrichments containing filters through which 500 ml of water was passed; –, no growth).

Sample	DIRB		SRB		MB		FB	AB
	om	H_2	om	H_2	om	H_2	om	H_2
DB-11	–	–	1	1	2	2	1	4
DC-06	+	+	2	4	+	1	4	4
Jw-1	+	–	3	4	1	4	1	4
Jw-2	+	–	2	+	1	2	1	4
Jw-3	+	–	1	1	1	1	2	4
Pr-3	+	–	4	3	2	4	3	3
Pr-4	–	–	+	1	2	4	2	4
Pr-5	–	–	1	1	1	4	3	4

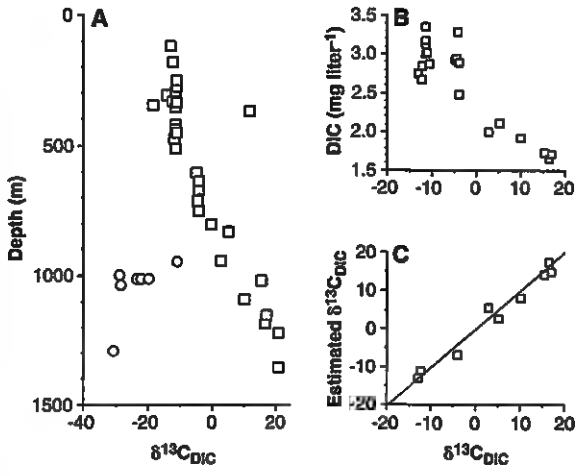

Fig. 3. Stable carbon isotopic signatures of confined aquifer samples. (**A**) Compositional variation with depth (O, high-sulfate samples). $\delta^{13}C_{DIC}$ generally increases with depth, which suggests carbon fixation by methanogenic bacteria, but in high-sulfate ground waters, $\delta^{13}C_{DIC}$ decreases, which suggests oxidation of biologically fixed carbon by sulfate-reducing bacteria. Compositions from less than 500 m below the land surface are relatively uniform and apparently have been little affected by methanogenesis. (**B**) $\delta^{13}C_{DIC}$ values increase with decreasing DIC. (**C**) $\delta^{13}C_{DIC}$ values of ground waters predicted by a fractionation model (*14, 37*) are nearly identical to measured values ($y = 1.009x + 0.005$, $r^2 = 0.928$). Results are plotted for samples that originated at depths below ~300 m and for which DIC concentration and $\delta^{13}C_{DIC}$ were known.

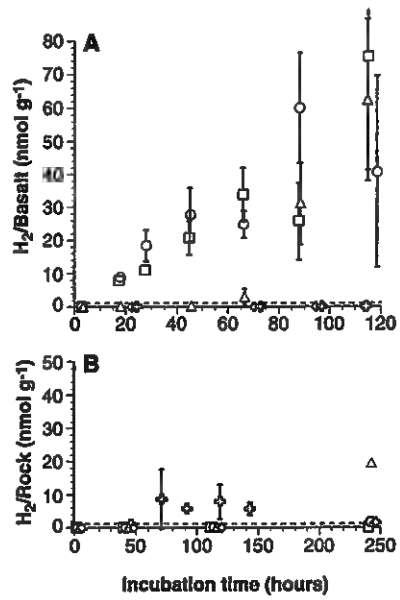

Fig. 4. Hydrogen gas production by in vitro basalt-water reactions. (**A**) Hydrogen produced by three basalt samples at 22°C [□, Umtanum Ridge basalt (*24*); O, unweathered Cerro Negro basalt (from near Mount Taylor, NM); △, Snake River Plain basalt (from near Idaho Falls, ID); ✣, Umtanum basalt control with no buffer; ◇, buffer control with no basalt]. (**B**) Hydrogen production by other rocks [□, sandstone; O, highly weathered Cerro Negro basalt dike 1; △, highly weathered Cerro Negro basalt dike 2; ◇, highly weathered Cerro Negro basalt dike 3; ✣, a granitic sample (from the Jurassic Ponder Pluton, British Columbia, Canada) containing ~10 volume % hornblende and 11 volume % biotite]. The data are the mean ± SD of three or more replicates; dashed lines indicate detection limits.

rapid H_2 evolution occurred (Fig. 4A) (*25*). The reactions occurred at room temperature and pressure and were completely inhibited by air (*26*). Similar results were obtained with basalt samples collected and processed on different days and with basalt samples collected from different regions. No H_2 was produced from basalt alone or buffer solution alone in control experiments, which indicated that the results were not attributable to the liberation of gases trapped in vesicles. Moreover, no H_2 was produced in assays with low-reactivity materials such as sandstone (Fig. 4B), and only trace amounts of H_2 were produced after extended incubation with highly weathered basalt samples, which should be depleted of reactants. A marked lag time consistently preceded H_2 evolution in a low-glass sample of Snake River Plain basalt, which then proceeded at the fastest measured rate; this finding suggests that more than one mineral phase is involved in this phenomenon. That is, some initial reactions (for instance, lowering the solution redox potential) by one phase may be required to establish favorable conditions for H_2 evolution by another phase. A granitic sample produced small amounts of H_2 after a 50-hour lag time, which indicates that at least some other rocks that contain ferrous silicate can also react to produce H_2.

If H_2-producing reactions occur in situ in the CRB and in similar crystalline rock formations, this phenomenon represents a widespread potential energy source for microbial metabolism in the subsurface. We speculate that unreacted mineral phases are exposed to ground water by ongoing microfracturing, the slow advancement of weathering fronts, and microbiologically enhanced weathering. These processes should result in regional production of potential microbial electron donors.

To determine whether basalt-water reactions alone could support microbial metabolism, we prepared a series of microcosms that contained only sterile crushed basalt and CRB ground water with its entrained microbial flora (*27*). A representative high-methane, low-sulfate ground water and a low-methane, high-sulfate ground water were used. As shown in Table 3, microorganisms proliferated in these microcosms. Most functional groups initially increased in number by several orders of magnitude, but with increasing time, autotrophic organisms maintained high numbers while heterotrophic microorganisms declined. This was true even in the high-sulfate microcosms where electron acceptors were not limiting. No microorganisms could be detected in microcosms that contained basalt and sterile buffer; in microcosms not containing basalt, no viable microorganisms could be detected after 24 weeks. Although microcosms might be subject to a "bottle effect," such an artifact would affect heterotrophs and autotrophs equally. The data suggest that growth and survival was promoted by geochemical H_2 production from basalt and ground water.

The prevalence of AB in these cultures seems unusual. Nearly all bacteria known to be capable of growing as AB can also grow as FB, although this was not indicated in our data. The role of $H_2 + CO_2$ acetogenesis in nature is not clear (*28*); it yields even less energy than methanogenesis, and MB should dominate AB in competition for H_2. In most sediments, AB activity accounts for only a few percent of in situ electron flow (*28*). We hypothesize that AB may successfully compete in the CRB aquifers because of the high H_2 concentrations. In this system, AB, as well as SRB and MB growing on $H_2 + CO_2$, could function as primary producers of organic matter.

The above results lead us to propose that a SLiME is largely responsible for the dissolved methane within the CRB. Because energy sources and inorganic nutrients both can be supplied by geochemical means in situ, microorganisms in SLiMEs can potentially persist in the deep terrestrial subsurface indefinitely. Several investigators (*29*, *30*) have speculated about possible lithoautotrophy in the subsurface. We have shown evidence for such a system and for a purely geochemical energy source. Furthermore, high temperatures or upwelling of geothermal fluids (*29*, *30*) are not required. The occurrence of SLiMEs may be widespread where appropriate mineralogic and physical conditions exist. For instance, extensive microbial populations have been reported in granitic aquifers in Sweden and Canada (*31*); these findings might be explained by reactions such as those in Fig. 4B. Anomalous concentrations of H_2 and CH_4 that include a bacteriogenic component have been reported in granitic terrains throughout the Canadian and Fennoscandian shields (*32*).

Autotrophic metabolism coupled to mineral weathering is an unusual geomicrobiological relation that has broad implications for microbial ecology. No other ecosystem is currently known to exist independently of past or present photosynthesis (*33*). SLiMEs conceivably provide a model for the existence of contemporary life on Mars because basalt, liquid water, and bicarbonate are believed to be present in the martian subsurface (*29*, *34*). SLiMEs may also provide a model for how surface organisms could have lived on Earth before the evolution of photosynthesis and the development of an oxidizing atmosphere about 2.8 billion years ago (*35*).

Table 3. Numbers of microorganisms in basalt–ground-water microcosms as a function of incubation time. The data are logarithms of numbers of organisms per milliliter belonging to various functional groups by enrichment series. The maximum detection limit was 10^4 organisms (–, no growth). DB-11 microcosms contained low-sulfate ground water; DC-06 microcosms contained high-sulfate ground water.

Microcosm (well)	Time (days)	DIRB		SRB		MB		FB	AB
		om	H_2	om	H_2	om	H_2	om	H_2
DB-11	0	–	–	1	1	2	2	1	4
	14	1	1	–	–	4	4	4	4
	49	1	1	–	–	4	4	4	4
	77	1	1	–	–	2	4	3	4
	238	–	2	–	–	–	4	–	4
	350	–	2	–	–	–	4	–	4
DC-06	0	–	–	2	4	–	1	4	4
	14	1	1	4	4	–	–	4	4
	49	–	–	3	4	–	–	3	4
	77	1	1	2	4	–	–	2	4
	238	1	–	2	4	–	–	2	4
	350	–	–	2	4	–	–	2	4

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7. Functional groups of bacteria were enumerated as described in (*6*), except that decimal dilution series

were used to estimate numbers of organisms and additional enrichment series were prepared for each electron acceptor. In the additional series, hydrogen was provided as the sole electron donor by pressurizing the headspace with an oxygen-free 50:50 mixture of H_2 and CO_2 at 2 bar.

8. Wells, either artesian or pumped, were purged for at least three well volumes and until field measurements (pH, Eh, conductivity) stabilized. Sample containers were thoroughly flushed with formation water and then aseptically filled and tightly sealed with butyl stoppers. This procedure was not as reliable as aseptically obtained core samples from the aquifers would have been; however, the agreement of microbiological results with regional ground-water signatures indicated that the results were representative of in situ aquifer communities.
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11. D. R. Lovley and S. Goodwin, *Geochim. Cosmochim. Acta* **52**, 2993 (1988). Five sedimentary interbeds containing 0.01 to 0.6% organic carbon are present in our study area and may contribute to the ecology of the upper parts of the CRB. We incubated samples of each interbed with CRB ground water and its entrained microorganisms in anaerobic microcosms for up to 2 years to determine whether organic matter in interbeds could promote microbial fermentation and subsequent production of biomass and hydrogen. Sediments from two interbeds could promote the survival and growth of typical anaerobic communities, although, as expected, elevated H_2 concentrations did not result. These interbeds are minor components of the CRB and are unlikely to be responsible for the amount of biomass we observed. In any case, in our study area, high concentrations of both H_2 and bacteria (*6*, *36*) were observed hundreds of meters below the deepest interbed. Thus, fermentation of organic matter in interbeds cannot explain the regional observations discussed above.
12. Artificially high hydrogen measurements can result from the use of stainless steel submersible pumps during sampling [D. R. Lovley, F. H. Chapelle, J. C. Woodward, *Environ. Sci. Technol.* **28**, 1205 (1994)]; because 36% of our samples were obtained with these pumps, individual values from the data set should be accepted with caution. The data did not show any correlation of H_2 concentration with depth sampled, production rate, or sampling method, and hence H_2 was likely not a sampling artifact.
13. Isotopic signatures are expressed in standard notation. The departure of the sampled $^{13}C/^{12}C$ ratio (R) from that of the standard material (Pee Dee belemnite), in parts per mil, is calculated according to

$$\delta^{13}C_{DIC} = \left(\frac{R_{sample} - R_{standard}}{R_{standard}}\right) \times 1000$$

14. Predicted $\delta^{13}C_{DIC}$ values were calculated with a Rayleigh fractionation model that conformed to the zero input–one output model of Wigley *et al.* (*37*),

$$(\delta^{13}C_{DC} + 1000) = (\delta^{13}C_{DIC_0} + 1000)\,(mC/mC_0)^{\alpha-1}$$

where mC/mC_0 is the ratio of measured DIC to initial DIC and α is a fractionation factor. Carbon removed from ground water as methane was assumed not to further interact with DIC. Initial DIC (C_0) and $\delta^{13}C_{DIC_0}$ were fixed at values predominant within the aquifers less than 500 m below the land surface (2.75 mmol liter^{-1} and −13 parts per mil, respectively) and α was fixed at 1.060. In nature, fractionation of methane relative to DIC varies from −40 to −90 parts per mil (*38*), corresponding to α values of 1.040 to 1.090. The fraction of DIC remaining for each calculation was determined as mC/mC_0 and the resultant calculated $\delta^{13}C_{DIC}$ was compared with the directly measured value (Fig. 3).
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22. A well-studied example of abundant H_2 that may have resulted from mafic rock-water interaction occurs in Kansas. H_2 abundances in excess of 30 volume % were found to have originated through shallow low-temperature water-rock interaction; although kimberlites are known to occur approximately 50 km from wells containing H_2, no proximate ultramafic rocks have been identified (*18*) [E. D. Goebel and G. A. M. Dreschhoff, *Oil Gas J.* **82**, 215 (1984); R. M. Coveney Jr., E. D. Goebel, E. J. Zeller, G. A. M. Dreschhoff, E. E. Angino, *Am. Assoc. Pet. Geol. Bull.* **71**, 39 (1977)]. Serpentinization reactions involving mafic igneous rocks known to exist in the Precambrian basement near the wells have been suggested as the source of H_2. These authors speculated from this association that H_2-containing gases may be more common than previously recognized, because of generation by weathering of mafic minerals in the subsurface; our data tend to confirm this inference. H_2 evolution from ground hedenbergite, olivine, and dunite in a poorly controlled experiment was reported by V. I. Molchanov, *Dokl. Akad. Nauk SSSR* **182**, 192 (1968).
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25. Reaction conditions for data shown were: temperature, 22°C; headspace, O_2-free N_2 at 1 bar; basalt sample, 5 g, particle size <0.25 mm; solution, 5 ml oxygen-free 1 mM sodium phosphate buffer, pH 6.0. Basalt aliquots were placed in 20-ml glass pressure tubes filled with N_2 gas and sealed with gas-impermeable butyl-rubber stoppers and aluminum crimp seals [W. E. Balch and R. S. Wolfe, *Appl. Environ. Microbiol.* **32**, 781 (1976)]. To avoid addition of exogenous H_2, we did not use the usual heated copper catalyst. Tubes were autoclaved for 30 min at 121°C, allowed to cool to room temperature overnight, and reautoclaved to obtain sterile samples. The autoclaving process was not required for H_2 production (T. O. Stevens and J. P. McKinley, data not shown) but was used to reduce variability resulting from the growth of random incidental microorganisms and to rule out biological contributions to hydrogen production. Small amounts of H_2 produced during autoclaving were removed by flushing sterile tubes with N_2 gas introduced through a 0.2-μm syringe filter and a hypodermic needle while venting through another needle. Sterile anaerobic buffer was prepared by boiling under N_2 gas and cooling to room temperature before dispensing into N_2-flushed pressure tubes, sealing, and autoclaving. Experiments were initiated by transferring an aliquot of buffer into a tube of basalt with a sterile, N_2-flushed, hypodermic needle and syringe. Hydrogen was measured by gas chromatography of a headspace sample, with either a Hewlett-Packard 5890-II gas chromatograph with a thermal conductivity detector (for micromolar concentrations) or a Trace Analytical RGA3 reduction-gas analyzer with a mercury-reduction detector (for nanomolar concentrations). Routine controls included basalt samples to which no buffer was added and buffer aliquots with no basalt sample.
26. T. O. Stevens and J. P. McKinley, data not shown.
27. Microcosms were sterile 160-ml serum bottles with butyl-rubber stoppers and aluminum seals. Each contained 50 g of autoclaved basalt chips (size <4 mm) and was filled with ground water at the wellhead, leaving no headspace, and sealed with sterile butyl stoppers. Microcosms were incubated in the dark at room temperature for up to 1 year and were sampled periodically by sacrificing individual microcosms. Assays were performed as described in (*7*).
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Large Arctic Temperature Change at the Wisconsin-Holocene Glacial Transition

Kurt M. Cuffey,* Gary D. Clow, Richard B. Alley, Minze Stuiver, Edwin D. Waddington, Richard W. Saltus

Analysis of borehole temperature and Greenland Ice Sheet Project II ice-core isotopic composition reveals that the warming from average glacial conditions to the Holocene in central Greenland was large, approximately 15°C. This is at least three times the coincident temperature change in the tropics and mid-latitudes. The coldest periods of the last glacial were probably 21°C colder than at present over the Greenland ice sheet.

The Greenland Ice Sheet Project II (GISP2) deep ice core has yielded a remarkable history of the oxygen isotopic composition of central Greenland snowfall [$\delta^{18}O$ of ice (*1*)] that extends through the last glacial period (*2*). The nearby Greenland Ice Core Project (GRIP) record (*3*) is essentially identical for ice formed after the 110,000-year-old Eemian interglacial, and both are similar to isotope histories obtained in other Greenland cores, giving confidence that these cores record aspects of regional climate (*4*). Using both empirical data (*5*) and physical models for isotope fractionation (*6*), paleoclimatologists have interpreted $\delta^{18}O$ to be a measure of environmental temperature T at the core site, through a simple relation that we call the isotopic paleothermometer: $\delta^{18}O = \alpha T + \beta$, where α and β are constants. There are two obstacles to making this interpretation sound. First, the coefficients α and β are not known a priori (*7–9*) because many factors in addition to local environmental temperature affect isotopic composition. These include changes in sea-surface composition and temperature (*10*), changes in atmospheric circulation (*11*), changes in cloud temperature, which may be different from changes in surface temperature (*12*), changes in the seasonality of precipitation (*13*), and postdepositional isotopic exchange in the snowpack (*14*). Second, all of these factors may vary through time in such a way that a single, linear relation between $\delta^{18}O$ and T is inappropriate. Thus, there is strong motivation to seek paleotemperature information that is entirely independent of isotopic history (*15*, *16*) to calibrate the paleothermometer. We have obtained such information by measuring temperature at depth in the ice sheet, and we use this information to evaluate α and β.

During the summer of 1994, one of us (G.D.C.) measured temperature in the 3044-m-deep GISP2 core hole from 70 m below the surface to the base of the ice sheet. At that time, the thermal perturbation from drilling had decayed to less than 0.04°C, so the temperature in the borehole matched the temperature in the surrounding ice sheet at this accuracy and better (*17*). To determine the coefficients α and β in the isotopic paleothermometer, we used the GISP2 $\delta^{18}O$ record and an initial guess for α and β to specify a 100,000-year history of environmental temperature. We then calculated subsurface temperatures using T as the forcing function on the upper surface of the ice sheet in a linked heat- and ice-flow model. Finally, we adjusted α, β, and the geothermal heat flux from the underlying bedrock, using the Levenberg-Marquardt method (*18*) to minimize the mismatch between modeled and measured subsurface temperatures. For this purpose we defined the mismatch index J as a weighted integral over ice depth z of the squared difference between modeled and measured subsurface temperatures (M and Θ, respectively)

$$J \equiv \int \frac{[M(z) - \Theta(z)]^2}{[\sigma_t^2(z) + f\sigma_D^2(z)]}\, dz \qquad (1)$$

Here σ_D and σ_t are weighting functions that assign relative importance to misfit in various parts of the borehole: σ_D assigns more weight to the upper part of the borehole, where the temperature does not depend on poorly known ice dynamical quantities, and σ_t assigns more weight to the lower part of the borehole, where the temperature depends on longer intervals of the surface temperature history. The parameter f, which is adjustable, controls the trade-off between these opposing weighting schemes (*19*). The solution that minimizes J is nonunique because we are free to choose f.

The heat-flow component of our model is a numerical solution to the advection-diffusion equation with heat sources (*20*). The model is one-dimensional (vertical), with a movable upper boundary to allow changes in the ice sheet thickness. These changes, and the vertical ice velocity responsible for heat advection, are calculated with standard glaciologic assumptions for flow on the flank of an ice divide with simple parameterizations to account for two-dimensional effects. In our model, the ice sheet responds to local changes in snow accumulation rate, surface temperature, and ice crystal fabric and to distant changes in ice margin position (*21*). To calculate vertical heat advection most confidently, and to account for two-dimensional effects on ice particle paths, we tuned the vertical velocity so that the modern depth-age scale matched our model within a small tolerance. Because of this tuning, our conclusions are insensitive to poorly known aspects of the ice dynamics. The GISP2 depth-age scale was determined by annual layer counts to about 40,000 years ago (40 ka) and by correlation to the ocean-core time scale (SPECMAP) through analysis of the oxygen isotopic composition of O_2 gas for older ice (*22–24*).

The snow accumulation rate history $b(t)$ exerts a dominant control on ice sheet thickness and vertical ice flow, and hence vertical heat advection. For the most recent 35,000 years, we derived $b(t)$ from the layer thickness measurements of Meese and others (*23*), corrected for ice-flow thinning. Our correction uses strain calculations as done previously (*24*, *25*), but we also included the dependence of layer thinning on the thickness history of the ice sheet (*26*, *27*), which in turn depends on the temperature history. Before 35 ka in the model

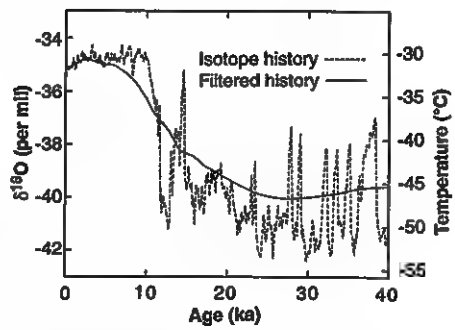

Fig. 1. The central Greenland $\delta^{18}O$ history for the most recent 40,000 years. The smooth curve results when this history is filtered to mimic the thermal averaging in the ice sheet (*45*). All temperature histories that give this same curve when filtered are indistinguishable to borehole thermometry (*29*). The right axis shows our calibrated temperature scale.

K. M. Cuffey, Department of Geological Sciences, Box 351310, University of Washington, Seattle, WA 98195, USA.
G. D. Clow, U.S. Geological Survey, Mail Stop 975, Menlo Park, CA 94025, USA.
R. B. Alley, Earth System Science Center and Department of Geosciences, The Pennsylvania State University, University Park, PA 16802, USA.
M. Stuiver, Quaternary Isotope Laboratory and Department of Geosciences, Box 351310, University of Washington, Seattle, WA 98195, USA.
E. D. Waddington, Geophysics Program, Box 351650, University of Washington, Seattle, WA 98195, USA.
R. W. Saltus, U.S. Geological Survey, Mail Stop 964, Denver Federal Center, Denver, CO 80225, USA.
*To whom correspondence should be addressed.

runs, we calculate $b(t)$ from the oxygen isotopes using a linear correlation between $\delta^{18}O$ and our $b(t)$ for the most recent 35,000 years (*28*).

Because heat diffusion damps high-frequency temperature changes as they propagate from the surface down into the ice sheet (thermal averaging), information on rapid environmental temperature changes in the past is poorly retained by the present-day temperature field $\Theta(z)$ in the ice sheet (*29*). Thermal averaging is more extensive for older climatic events. In contrast, the GISP2 $\delta^{18}O$ record retains information about rapid climatic changes. If we degrade the $\delta^{18}O$ record so that it retains only the age-dependent low-frequency content that can be recovered from the present-day temperature field (Fig. 1), then the abrupt termination of the Younger Dryas, the Younger Dryas itself, and the Bølling/Allerød period become minor features of the history, and earlier interstadial events are no longer evident. Thus, our isotope calibration is sensitive mainly to the long warming from full glacial conditions to the Holocene, and to Holocene temperature changes (*30*).

We find the optimal linear paleothermometer to be $\delta^{18}O = 0.327T - 24.8$ if we assume ice dynamics are well known ($f = 1$; refer to Eq. 1), and $\delta^{18}O = 0.335T - 24.5$ if we assume ice dynamics are poorly known ($f = 1000$) (*31*). Using these calibration constants, we find a remarkably good fit between the temperatures measured in the borehole (Fig. 2) and the corresponding modeled temperatures; the model accounts for 99.88% of the variance of the measured profile relative to steady-state. This is strong evidence that $\delta^{18}O$ is indeed a faithful proxy for long-term average temperature at this site. There is no better explanation for the success of such a simple calibration, given the small number of free parameters in the inversion.

However, the fit is not perfect. For instance, the isotope record underpredicts the magnitude of cooling in the late Holocene. If we allow some time variation of α (*32*), J is minimized with $\alpha \approx 0.33$ per mil °C^{-1} (*33*) during the deglacial transition. For much of the Holocene the optimal value for α is 0.25 per mil °C^{-1}. In the most recent several centuries, for which higher frequency climate changes are resolved by the borehole temperatures, α becomes larger (0.46 per mil °C^{-1}) and closer to the value inferred from modern temperature records (*33*). Our result shows that the general circulation model of Jouzel *et al.* (*8*) can provide better estimates of past values of α than the value of 0.60 to 0.67 per mil °C^{-1} derived from the modern spatial correlation; they predict $\alpha \approx 0.43$ per mil °C^{-1} for the deglacial transition by linking changes in atmospheric circulation and source temperature to a physical model of isotope fractionation (*34*).

The low value of α that we find for the deglacial transition is well-constrained (Fig. 3) and insensitive to changes in ice dynamical parameters (Table 1). The average temperature difference between the Wisconsin Glaciation and the Holocene is therefore large (Fig. 1), 14° to 16°C, and the coldest periods of the last glacial were probably 21°C colder than at present (Fig. 1). The climatic deglacial temperature change (at constant elevation) may be 1° to 2°C larger than this because the Greenland ice sheet was probably thinner during the glacial as a result of a substantial reduction in accumulation rate. Geologic evidence suggests that the margins of the ice sheet retracted by about 100 km during the Wisconsin-Holocene transition (*35*). Using this value, and assuming a symmetrical retreat of east and west margins, we estimate that the ice sheet thickened by 250 m from the last glacial maximum to the present, and at least 100 m from average glacial conditions to the present (*36*).

Table 1. Sensitivity of $\alpha = d(\delta^{18}O)/dT$ to changes in ice dynamical quantities that are poorly known or uncertain (*47*). The constant α is most sensitive to adjustments of the age-depth relation (the time scale); however, even this sensitivity is minor. The 2% uncertainty in age at the Younger Dryas termination (1680 m in depth) is an estimate by Alley *et al.* (*24*).

Model adjustment	α (per mil °C^{-1})	ΔT (°C)	
		Average glacial to Holocene	Coldest glacial to Holocene
No marginal retreat	0.327	15.3	21.4
Initial temperature 4°C warmer	0.328	15.2	21.3
Initial temperature 4°C colder	0.328	15.2	21.3
No north-south spreading	0.328	15.2	21.3
Use uncorrected $b(t)$	0.328	15.2	21.3
No fabric evolution	0.327	15.3	21.4
Age reduced by 2% at 1680 m and by 20% at 2800-m depth	0.341	14.7	20.5
Age increased by 2% at 1680 m and by 20% at 2800-m depth	0.313	16.0	22.4

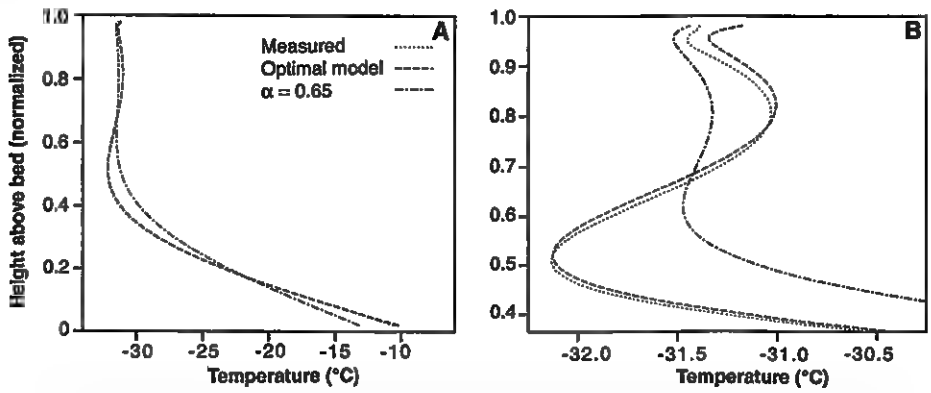

Fig. 2. Comparison of measured and modeled temperatures within the ice sheet, as functions of height above the ice sheet bed (normalized to the thickness of the ice sheet). **(A)** The full ice sheet thickness. At this scale, the measurements and the optimal model results are indistinguishable. **(B)** The upper part of the ice sheet. The Little Ice Age, the mid-Holocene warmth, and the cold glacial are immediately evident in the temperature profile, as they are in the GRIP hole (*46*). The temperature increases considerably toward the bed because of geothermal heating. The best possible fit with the approximate modern spatial value of $\alpha = 0.65$ per mil °C^{-1} (*5*) is a poor match to the data.

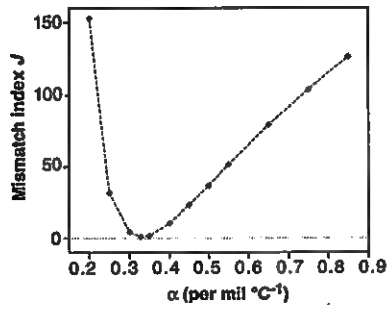

Fig. 3. The mismatch J between modeled and measured borehole temperature profiles, normalized to its minimum value, as a function of α. The well-defined minimum shows the location of the optimal value for α, 0.327 per mil °C^{-1}. To produce this curve, we chose values for α, then inverted for β and the geothermal flux to optimize the fit.

Recent estimates of the Wisconsin-to-Holocene warming in the low mid-latitudes are 4° to 6°C. This result is based on a variety of methods, including snow line depression studies (*37*), palynology (*38*), noble gas paleothermometry applied to ground water (*39*), and stable-isotope paleothermometry applied to coral reefs (*40*, *41*). The ~8°C temperature change commonly inferred from ice-core isotopic records (*37*), including those from the new GISP2 and GRIP cores (*2*, *4*), using the modern spatial value for α of 0.60 to 0.67 per mil °C^{-1}, is only slightly larger than recent estimates from the tropics. By contrast, we have shown that the temperature change in central Greenland was three to four times larger than that in the tropics, a result that is consistent with borehole temperature analyses at Dye 3 in southern Greenland (*42*). Many models have suggested that initially minor changes in global temperature will be magnified in the Arctic, with possibly major consequences for sea level and planetary albedo (*43*). Our data not only confirm that such amplification happened in the past but also show this amplification to be larger than generally thought.

REFERENCES AND NOTES

1. The value of $\delta^{18}O$ in units of per mil is defined as $\delta^{18}O = 1000[(R_{sample} - R_{standard})/R_{standard}]$, where the ratio $R \equiv [^{18}O]/[^{16}O]$ (brackets denote concentration), and the standard R is that for mean ocean water.
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9. The parameters α and β are usually derived from linear correlation of mean annual temperature and isotopic ratio with data from different sample sites across the surface of an ice sheet. The assumption that this modern spatial relationship is the same as the temporal relationship at a single site is known to be incorrect in some cases; see, for example, D. A. Peel, R. Mulvaney, B. M. Davison, *Ann. Glaciol.* **10**, 130 (1988).
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17. Temperatures were measured in the fluid-filled section of the borehole with an updated version of the system described in G. D. Clow *et al.*, *U.S. Geol. Surv. Open-File Rep. 95-490* (1995). Under the environmental conditions at GISP2, the system sensitivity is 0.00014°C; the accuracy of the measurements traced to the National Institute of Standards and Technology is ~0.0045°C. Temperature measurements were acquired every 2 s while lowering a custom sensor with 20 thermistor beads down the hole at ~6 cm s^{-1}. Instrumental noise during this experiment was limited to less than 0.001°C. Data from the moving sensor were then deconvolved to find the stationary borehole temperatures by the methods described in R. W. Saltus and G. D. Clow, *U.S. Geol. Surv. Open-File Rep. 94-254* (1994). The precision of the completely processed data is estimated to be better than 0.001°C for wavelengths greater than about 6 m. Drilling activities between 1990 and 1993 disturbed the temperatures in the ice surrounding the borehole. Comparison of temperature measurements acquired in 1994 and 1995 in the deep borehole, and temperatures acquired to 163 m in a nearby air-filled hole, shows that the magnitude of the disturbance in 1994 was about 0.04°C near the top of the fluid-filled section of the main borehole. This disturbance diminished to about 0.001° to 0.002°C at 400 m in depth and remained at this level to the bottom of the hole. Because the disturbance is largely restricted to the upper part of the borehole, its effect on our inferred deglacial temperature change is negligible (about 1.5% of the temperature change).
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19. Poorly known ice dynamical quantities such as ice rheology, ancient snowfall rates, ice sheet geometry changes, and initial temperature constitute the major sources of uncertainty in calculating subsurface temperatures. This uncertainty is large deep in the borehole and small near the surface, suggesting that we should ascribe more importance to misfit in the upper part of the borehole: σ_D is a rigorous means of doing so. However, temperatures high in the borehole depend on only the most recent several centuries of the temperature history, whereas temperatures deep in the ice sheet depend on many tens of thousands of years of the temperature history. This suggests we should ascribe more importance to misfit in the lower part of the borehole, to find the isotopic paleothermometer that applies best to the whole isotopic history: σ_I is a rigorous means of doing so. We do not know what the most appropriate balance between these opposing weighting schemes should be; therefore, we introduce the trade-off parameter f and show that our result does not depend significantly on the choice of weighting scheme.
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21. The model has 130 to 150 control volumes in the ice sheet (depending on the thickness history) and extends 10 km into bedrock. The model was tested against analytical solutions for sinusoidal surface-temperature forcing and uniform ice velocity, with the time step chosen to give 0.001°C accuracy. The vertical velocity at a given level in the ice sheet equals the ice flux divergence beneath that level. The thickness changes at a rate equal to the imbalance between snowfall and flux divergence of the entire thickness. Changes in slope and slope gradient of the ice sheet are calculated by assuming that the ice sheet evolves through a series of steady-state geometries, except in the response to marginal forcing. For the latter, the ice sheet responds as a diffusive system in a manner that is consistent with the full two-dimensional model of R. B. Alley and I. M. Whillans [*J. Geophys. Res. C* **89**, 6487 (1984)]. At GISP2 it is not necessary to consider horizontal heat advection because the elevation difference between GISP2 and the ice divide is small. The position of the ice divide has probably changed through time [S. Anandakrishnan, R. B. Alley, E. D. Waddington, *Geophys. Res. Lett.* **21**, 441 (1994)]. As long as GISP2 has remained more than several ice thicknesses from the divide, the horizontal temperature gradient at depth has not been important [W. S. B. Paterson and E. D. Waddington, *Cold. Reg. Sci. Tech.* **12**, 99 (1986)].
22. The upper part of the ice core is dated by counting of annual layers (visible stratigraphy, particle counts, electrical conductivity, and oxygen isotopes) (*2*, *23*, *24*); M. Ram and M. Illing, *J. Glaciol.* **40**, 504 (1994); K. C. Taylor *et al.*, *Nature* **366**, 549 (1993). For the lower part of the core, see M. Bender *et al.*, *ibid.* **372**, 663 (1994).
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30. If borehole temperature measurements are sufficiently accurate, even short or ancient features of the temperature history can be detected (*29*, *44*). However, their contribution to the modern temperature signal is negligible, so they do not affect our isotopic thermometer calibration.
31. Setting high f means we do not try to match the measured temperatures deep in the borehole. By contrast, in the sensitivity tests presented later (Table 1), we do seek a match at all depths and examine how our result depends on specific inputs.
32. On the basis of thermodynamics arguments and a changing relation over time between $\delta^{18}O$ (*2*) and snow accumulation rates (*23*) from the GISP2 ice core, M. Stuiver, P. M. Grootes, and T. F. Braziunas [*Quat. Res.*, in press] inferred that α had changed in response to climate changes, with smaller values during the deglacial transition.
33. We find six parameters—that is β, the geothermal flux, and values for α in each of four time intervals—that minimize J (with $f = 100$) using the Levenberg-Marquardt method. We find α to be 0.33 per mil °C^{-1} before 8 ka, 0.25 per mil °C^{-1} for the early Holocene, 0.25 per mil °C^{-1} for the late Holocene cooling, and 0.46 per mil °C^{-1} for the Little Ice Age to the present. We invert for only one value of β because the others are chosen to ensure continuity of the temperature history. Two independent studies [(*7*) and C. A. Shuman *et al.*, *J. Geophys. Res. D* **100**, 9165 (1995)] also found relatively high values of α for recent times. This trend in α could reflect a changing climate system (*32*); it could also indicate that $\delta^{18}O$ is more sensitive to temperature changes over years to centuries than to temperature changes over periods of millennia or longer. The Cuffey *et al.* (*7*) α value is slightly higher than ours (0.53 versus 0.46 per mil °C^{-1}) because their measurements extend 40 m closer to the ice sheet surface and hence are weighted more heavily to recent temperature changes [see figure 3 in (*7*)].
34. Changes in seasonality of snow accumulation may contribute to the difference between the spatial and temporal gradients [(*13*) and P. J. Fawcett, A. M. Agustsdottir, R. B. Alley, *Eos* **76** (spring suppl.), 177 (1995)]. We used raw isotopic data from the ice core, with no adjustment for changes in source-water isotopic composition, whereas Jouzel *et al.* (*8*) assumed that source-water changes equaled the effect of ice-volume changes on mean oceanic composition, but removed these source-effects from their calculated last glacial maximum $\delta^{18}O$ when they estimated the spatial gradient α. Recalculation of the Jouzel *et al.* (*8*) temporal gradient with the snowfall isotopic change uncorrected for source effects would yield an even closer match to our result.
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from all extant models have large uncertainties because changes in ice marginal position, accumulation rate, and effective ice softness are poorly known. Our thickness history is consistent with the detailed layer thickness analysis of Bolzan *et al.* (*26*). However, we cannot be confident that this thickness increase implies a greater climatic temperature change, because the difference between cloud and surface temperatures also may have changed.
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41. Estimates of the tropical glacial-Holocene warming based on planktonic microfossil assemblages are considerably smaller [CLIMAP Project Members, *Geol. Soc. Am. Map Chart Ser. MC-36* (1981); (*40*)]. This discrepancy is not resolved, but a wider variety of evidence indicates ~5°C of warming.
42. Interpretation of borehole temperature data from Dye 3 must include strong horizontal heat advection, which has probably changed through time, and larger uncertainties in the accumulation history compared to GISP2. The GISP2 core has shown the glacial accumulation rate to be approximately one-third that of the Holocene (*24*). For this accumulation rate contrast, two analyses of Dye 3 temperature (*16*, *44*) indicate an approximately 14°C deglacial temperature change.
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48. This work was supported by the NSF (some material is based on work supported by a NSF Graduate Research Fellowship) and the U.S. Geological Survey. We thank the GISP2 Science Management Office, the 109th Air National Guard, the Polar Ice Coring Office, and GISP2 participants.

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Superplasticity in Earth's Lower Mantle: Evidence from Seismic Anisotropy and Rock Physics

Shun-ichiro Karato,* Shuqing Zhang, Hans-Rudolf Wenk

In contrast to the upper mantle, the lower mantle of the Earth is elastically nearly isotropic, although its dominant constituent mineral [(Mg,Fe)SiO_3 perovskite] is highly anisotropic. On the basis of high-temperature experiments on fabric development in an analog $CaTiO_3$ perovskite and the elastic constants of $MgSiO_3$ perovskite, the seismic anisotropy was calculated for the lower mantle. The results show that absence of anisotropy is strong evidence for deformation by superplasticity. In this case, no significant transient creep is expected in the lower mantle and the viscosity of the lower mantle is sensitive to grain size; hence, a reduction in grain size will result in rheological weakening.

Rheological properties have an important influence on the nature of flow in the deep interior of the Earth, but both laboratory and theoretical studies of deep mantle rheology have significant limitations. Quantitative measurements of the strength of materials under high pressures and temperatures are difficult, and no rheological measurements have been performed under lower mantle conditions. Neither the rheological constitutive relation (that is, the dependence of viscosity on stress or grain size or both) nor the absolute values of viscosity of the lower mantle are well constrained (*1*). The rheology of the Earth's deep interior can be inferred from laboratory data only after a large extrapolation in time scales, which introduces a significant amount of uncertainty. Similarly, theoretical estimates of rheological properties of the Earth's deep interior from time-dependent deformation are difficult to make because of (i) the poor sensitivity of the data to rheological properties of the deep portions of the Earth (*2*), (ii) the uncertainties in some key input parameters such as the melting history of ice sheets (*3*) in the analysis of the postglacial rebound, or (iii) the density-to-velocity conversion factor (*4*) in the analysis of the geoid.

One strategy to get around these difficulties is to combine seismological observations of anisotropy and laboratory studies of deformation-induced lattice preferred orientation [for example (*5*)]. The anisotropic structure of deformed materials depends on deformation mechanisms (and deformation geometry) [for a review, see (*6*)], and anisotropic structures can be observed seismologically as far down as the center (that is, inner core) of the Earth (*7*). Plastic deformation by diffusion or superplastic creep will result in an isotropic structure, whereas deformation by dislocation creep or twinning results in an anisotropic structure. Thus, although this approach will not provide direct estimates of the absolute values of viscosities, it provides information as to the rheological constitutive relation (stress or grain-size dependence of viscosity) and hence indirectly indicates rheological discontinuities or weakening associated with grain-size reduction (*5*, *8*).

Here we apply this strategy to the lower mantle. One of the most striking observations of the lower mantle is the absence of significant seismic anisotropy (*9*) even though the dominant mineral in the lower mantle, orthorhombic (Mg,Fe)SiO_3 perovskite, has significant elastic anisotropy (*10*, *11*). The absence of observed anisotropy in the lower mantle could be attributed to (i) chaotic convection; (ii) limited plastic deformation; (iii) the anisotropic structure in the lower mantle which happens to be such that, for seismic waves traveling nearly vertically [such as those used in seismological studies (*9*)], the amount of shear wave splitting is small (*12*); or (iv) the fact that the deformation does not result in an anisotropic structure. The first hypothesis means that the lower mantle materials could have anisotropic structure (possibly due to deformation by dislocation creep), but the scale of coherent deformation is much smaller than the length of typical seismic wave paths (for ScS or SKS) so that, on average, no appreciable anisotropy would be detected. This hypothesis is unlikely because seismic tomography indicates that the lower mantle structure is dominated by long wave length features (*13*), and a high viscosity of the lower mantle will make chaotic convection difficult to achieve (*14*). The second hypothesis is also untenable because seismic tomography indicates the presence of downgoing and upwelling currents in the lower mantle (*15*), and the Rayleigh number for the lower mantle is likely to exceed the critical value [see, for example, (*1*)]. Discrimination of the last two alternatives requires an investigation of the relation between the nature of deformation and seismic anisotropy in lower mantle materials.

The most direct data on this subject must ultimately come from high-pressure, high-temperature deformation experiments performed on polycrystalline (Mg,Fe)SiO_3

S.-i Karato and S. Zhang, Department of Geology and Geophysics, University of Minnesota, Minneapolis, MN 55455, USA.
H.-R. Wenk, Department of Geology and Geophysics, University of California, Berkeley, CA 94720, USA.
*To whom correspondence should be addressed.

perovskites. However, these experiments cannot be done with current technology. Meade *et al.* studied the preferred orientation of $(Mg,Fe)SiO_3$ perovskite under lower mantle pressures and at room temperature (*16*). However, as deformation mechanisms at the room temperatures used in their study are not likely to be the same as those operating at the high temperatures of the lower mantle, the relevance of their results to the seismic anisotropy in the lower mantle is not clear.

In our study, we used an analog material, $CaTiO_3$, to gain insight into the geodynamical implications of the observed seismic isotropy of the lower mantle. Use of an analog material is suitable in the study of deformation fabrics (the preferred orientation of crystals) which are mainly determined by the nature of slip systems and twinning that in turn are primarily controlled by crystal structure. $CaTiO_3$ is considered to be a good analog because most of the defect-related features that control the preferred orientation are similar to the features found in $(Mg,Fe)SiO_3$ perovskite (*17*). Karato *et al.* (*18*) found that diffusion creep dominates in $CaTiO_3$ perovskite under a wide range of conditions, and they suggested that diffusion creep might be a dominant mechanism of flow in the lower mantle. On the basis of these results, we prepared polycrystalline $CaTiO_3$ with different grain sizes (~8 μm and ~70 μm in diameter) and deformed them to large shear strains (up to ~300%) using a technique described in (*19*). The sample with ~8-μm grain size was deformed in the diffusion creep regime, and the other sample with ~70-μm grain size was deformed in the dislocation creep regime. We chose this mode of deformation because the likely deformation geometry in the mantle is simple shear rather than axial compression (*20*) and because the large strains necessary for texture studies will be easily achieved in this geometry but not in axial compression. The deformation conditions were P (pressure) = 300 MPa and T (temperature) = 1498 K (which is equal to 0.68 T_m, where T_m is the melting temperature), and $\dot{\varepsilon}$ (shear strain rate) ranged from 0.7×10^{-5} to 18×10^{-5} s^{-1} up to ~310% strain (*21*).

The fine-grained samples typically showed homogeneous deformation, whereas the coarse-grained specimens showed some heterogeneous deformation caused by dynamic recrystallization in which relict grains show a large elongation but newly formed recrystallized grains remain nearly equant shape. The coarse-grained samples showed somewhat asymmetrical grain shapes, often referred to as fish (*22*), which presumably result from the deformation of fine-grained recrystallized grains (Fig. 1A). In contrast, almost all the grains in the fine-grained specimens showed nearly equant shapes despite large strain (Fig. 1B). These observations, together with the observed small stress exponent [$\dot{\varepsilon} \sim \sigma$, where σ is stress (*18*)] and grain-size sensitivity at small grain sizes [$\dot{\varepsilon} \sim 1/d^2$, where d is grain size (*18*)], imply that deformation in the fine-grained specimens was by superplasticity, where grain boundary sliding contributes significantly to strain (*23*).

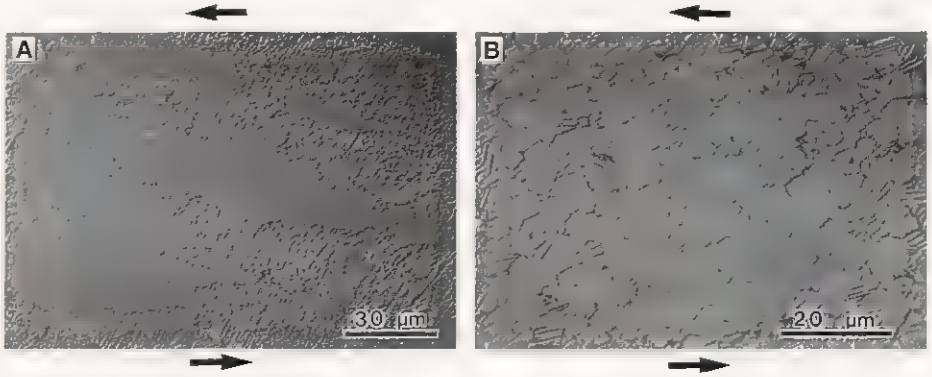

Fig. 1. Microstructures of deformed $CaTiO_3$ perovskite. Optical micrographs of polished sections after chemical etching taken with reflected light (with Nomarsky contrast). (**A**) A coarse-grained sample deformed in the dislocation creep regime [P = 300 MPa, T = 1498 K, $\dot{\varepsilon}$ (shear strain rate) = 18×10^{-5} s^{-1}, and ε (shear strain) = 310%]. (**B**) A fine-grained sample deformed in the superplastic regime (P = 300 MPa, T = 1498 K, $\dot{\varepsilon} = 4 \times 10^{-5}$ s^{-1}, and ε = 173%). Grain shape changes in the coarse-grained specimen follow approximately the strain ellipsoid. In contrast, almost no grain elongation is found in the fine-grained specimen. Numerous twin boundaries are seen in both specimens. Shear direction is east-west, and the sense of shear is indicated by arrows.

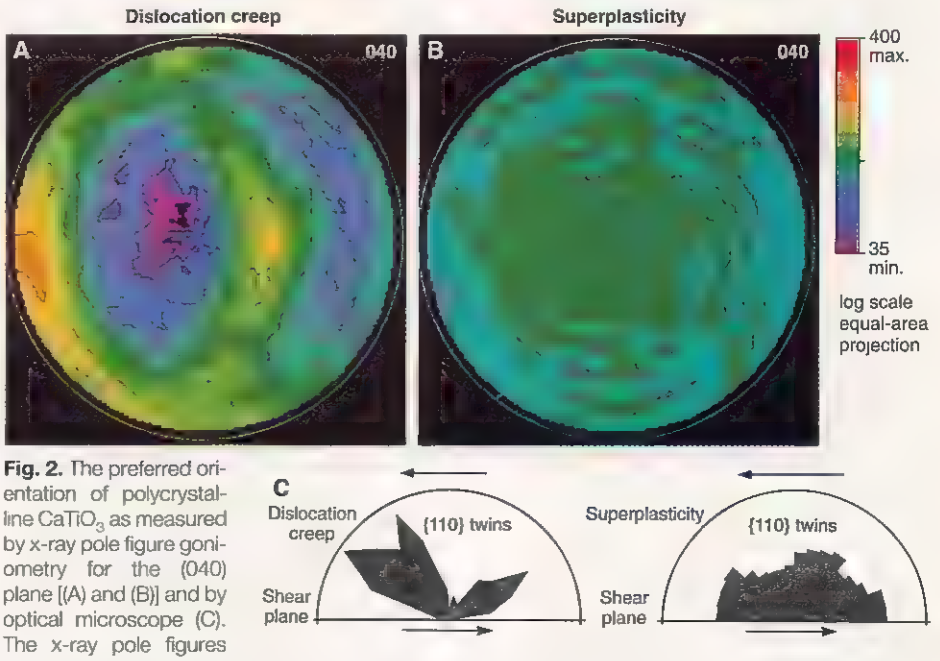

Fig. 2. The preferred orientation of polycrystalline $CaTiO_3$ as measured by x-ray pole figure goniometry for the (040) plane [(A) and (B)] and by optical microscope (C). The x-ray pole figures are plotted on the shear plane by using the upper hemisphere projection. The color-coded pole densities are related to a logarithmic scale that expresses pole densities in 100× multiples of a random distribution [a value of 100 (that is, green) means that the pole distribution is random]. (**A**) A coarse-grained sample deformed in the dislocation creep regime (T = 1498 K, $\dot{\varepsilon} = 18 \times 10^{-5}$ s^{-1}, and ε = 310%). (**B**) A fine-grained specimen deformed in the diffusion creep (superplastic) regime (T = 1498 K, $\dot{\varepsilon} = 5 \times 10^{-5}$ s^{-1}, and ε = 30%). Note the strong preferred orientation in the coarse-grained specimen and very weak preferred orientation in the fine-grained specimen. The strength of preferred orientation reaches nearly steady state at strains of ~50 to 70% in the dislocation creep regime; therefore, a large difference in the strength of preferred orientation between two sets of specimens is due mainly to the difference in deformation mechanisms and not by the difference in the magnitude of strain. This point is demonstrated in (**C**) where the orientation of the {110} twin boundaries is plotted for the two samples (one deformed in the dislocation creep regime at T = 1498 K, $\dot{\varepsilon} = 0.7 \times 10^{-5}$ s^{-1}, and ε = 30% and the other deformed in the superplasticity regime at T = 1498 K, $\dot{\varepsilon} = 4 \times 10^{-5}$ s^{-1}, and ε = 173%). The plots are on the plane perpendicular to the shear plane. About 2000 twin boundaries were measured.

We measured the fabric (lattice preferred orientation) of deformed samples using both an optical microscope equipped with a universal stage and an x-ray pole figure goniometer (Fig. 2) (*24*). Again, marked contrasts were found between fine-grained and coarse-grained specimens. The preferred orientation in coarse-grained samples was strong; the *a* axis became subparallel to the flow direction and the *b* axis perpendicular to the flow plane. In contrast, fine-grained specimens showed almost random preferred orientation, consistent with the absence of preferred orientation in superplastically deformed metals (*23*).

On the basis of the preferred orientation of individual grains measured with a universal stage, we calculated seismic wave velocities using the elastic constants of $MgSiO_3$ perovskite (*11*, *25*) and the program developed by Mainprice (*26*) (Fig. 3). For the ScS wave, whose pass length in the lower mantle is ~4000 km (~2000 km for SKS), the expected shear wave splitting for dislocation creep ranges from ~1 to ~36 s (0.5 to 18 s for SKS) depending on the geometry of flow. The observed small shear wave splitting (less than 0.2 s) is marginally consistent with a horizontal flow, but we consider that a horizontal flow is unlikely at least in the areas where shear wave splitting was studied (*27*). For a vertical flow, we expect shear wave splitting of ~20 s (~10 s for SKS), which is much larger than the observed values (less than 0.2 s). The preferred orientation in samples deformed by superplastic creep is much smaller; hence, weaker seismic anisotropy results (*28*).

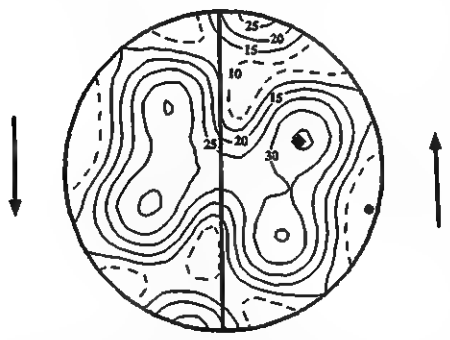

Fig. 3. Shear wave splitting of a perovskite aggregate deformed by dislocation creep, calculated from the preferred orientation of a coarse-grained $CaTiO_3$ and the elastic constants of $MgSiO_3$ perovskite. The magnitude of shear wave splitting is plotted on a plane perpendicular to the shear plane including the shear direction. The sense of shear is indicated by arrows. For vertical shear with a layer ~4000 km (~2000 km) thick, the flow direction is nearly parallel to the [100]-axis peak, and the expected shear wave splitting will be ~30 s (~15 s). For a superplastically deformed perovskite, the shear wave splitting is much smaller. ■, maximum of 36 S; ●, minimum of 1 s. Path length is 4000 km.

Therefore, we conclude that the observed small shear wave splitting in the lower mantle suggests that deformation there is by superplastic flow.

We have assumed that seismic anisotropy in the lower mantle is determined solely by that of perovskite. The secondary component, (Mg,Fe)O, is also anisotropic (*26*), and it may contribute to shear wave splitting. We suggest that the absence of shear wave splitting in the lower mantle implies that (Mg,Fe)O, if anything, should also deform by superplasticity.

Superplasticity is an important mechanism of deformation only under limited conditions. In particular, grain size must be maintained to be reasonably small (*23*). It is conceivable that a small grain size in the lower mantle is maintained by the presence of (Mg,Fe)O; otherwise, grain growth would result in deformation by dislocation creep.

There is strong evidence that deformation is by dislocation creep in the upper portions of the upper mantle (*5*). Thus, the rheological constitutive relation (that is, the dependence of viscosity on stress or grain size) is different between the upper and lower mantle. Viscosity in the superplasticity regime strongly depends on grain size. Therefore, a reduction of grain size in the lower mantle will result in significant rheological weakening (*18*, *29*). Also, the depth variation in viscosity of the lower mantle will depend on the depth variation in grain size. There will be no significant transient creep in the lower mantle. Thus, the rheology of the lower mantle inferred from the postglacial rebound will be free from the effects of transient creep and, therefore, should be identical to that estimated from geoid anomalies.

Effects of changes in rheological constitutive law on mantle convection could cause intermittent upwelling plumes from the boundary (*30*). Grain-size reduction associated with the phase transformation to the perovskite plus magnesiowustite assembly near the 660 km discontinuity will significantly weaken a subducted slab that penetrates into the lower mantle, which may cause it to deform or thicken, as has been seen in some seismic tomography (*15*).

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10. The dominant mineral of the lower mantle is (Mg,Fe)-SiO_3 perovskite [see, for example, A. E. Ringwood, *Geochim. Cosmochim. Acta* **55**, 2083 (1991)]. Both experimental [N. Funamori and T. Yagi, *Geophys. Res. Lett.* **20**, 387 (1993)] and theoretical [L. Stixrude and R. E. Cohen, *Nature* **364**, 613 (1993); R. M. Wentzcovitch, J. L. Martins, G. D. Price, *Phys. Rev. Lett.* **70**, 3947 (1993)] studies indicate that the perovskite [$(Mg,Fe)SiO_3$] assumes an orthohombic structure in most of the lower mantle conditions, although structural phase transformations to tetragonal or cubic (or some other unknown) structure might occur in the limited portions [G. H. Wolf and M. S. T. Bukowinski, in *High-Pressure Research in Mineral Physics*, M. H. Manghnani and Y. Syono, Eds. (Terra Publications Tokyo/American Geophysical Union, Washington, DC, 1987), pp. 313–331; Y. Wang, F. Guyot, R. C. Liebermann, *J. Geophys. Res.* **97**, 12327 (1992); C. Meade, H.-K. Mao, J. Hu, *Science* **268**, 1743 (1995)]. Because a significant portion of the lower mantle is likely to be occupied with an orthorhombic perovskite, the use of elastic constants and lattice preferred orientation for orthorhombic perovskite is justified as a first approximation. When the crystal structure changes to other symmetries, then both lattice preferred orientation and elastic constants will change, and as a result, the elastic anisotropy will also change. But some seismic anisotropy is expected in the dislocation creep regime because of a significant lattice preferred orientation due to anisotropic dislocation glide [for anisotropy in dislocation creep in cubic perovskite, see Z. Wang, S. Karato, K. Fujino, *Phys. Earth Planet. Inter.* **79**, 299 (1993)]. However, no appreciable effect is expected in the superplastic regime where no lattice preferred orientation will occur.
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higher temperatures in the lower mantle would be smaller. The physical basis for this argument is weak, however, because the nature of LPO is closely related to the geometry of slip systems and of twinning which is sensitive to temperatures, and there is no general relation between LPO and temperatures. In fact, less LPO is found under lower temperatures in olivine because of the complexities of the active slip systems [A. Nicolas and N. I. Christensen, in *Composition, Structure and Dynamics of the Lithosphere/Asthenosphere System*, K. Fuchs and C. Froidevaux, Eds (American Geophysical Union, Washington, DC, 1987), pp. 111–123]. This trend in olivine is inconsistent with the observation of Meade *et al.* and suggests that mechanisms other than dislocation glide (for example, fracturing) are responsible for the LPO observed in these experiments.

17. Defect-related properties that control the preferred orientation of minerals include slip systems and twinning mechanisms [see, for example, P. van Houtte and F. Wagner, in *Preferred Orientation in Deformed Metals and Rocks: An Introduction to Modern Texture Analysis*, H.-R. Wenk, Ed. (Academic Press, New York, 1985), pp. 233–258], both of which are similar between $CaTiO_3$ and $(Mg,Fe)SiO_3$ [N. Doukhan and J.-C. Doukhan, *Phys. Chem. Mineral.* **13**, 403 (1986); S. Karato, K. Fujino, E. Ito, *Geophys. Res. Lett.* **17**, 13 (1990); Y. Wang, thesis, State University of New York at Stony Brook (1991), p. 232].
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20. Deformation in the lower mantle will be dominated by a vertical motion associated with upwelling or downgoing plumes except near the bottom (that is, the D″ layer) and a possible boundary layer near the 660 km discontinuity. Thus, the dominant mode of deformation in the lower mantle will be vertical shear to a first approximation.
21. At this *P-T* condition, $CaTiO_3$ assumes an orthorhombic structure (space group *Pbnm*) that is the same as the structure of the $(Mg,Fe)SiO_3$ perovskite under most lower mantle conditions (*10*). The homologous temperature (that is, T/T_m) in the lower mantle is similar to these conditions (T/T_m = 0.6 to 0.7 in the shallow lower mantle [A. Zerr and R. Boehler, *Science* **262**, 553 (1993)]. Under these conditions, a fine-grained (~8 μm) $CaTiO_3$ will deform by diffusion (or superplastic) creep, whereas a coarse-grained (~70 μm) counterpart will deform by dislocation creep (*18*).
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24. X-ray measurements of the orientation of (040) lattice planes were made for both coarse- and fine-grained specimens in reflection and transmission geometry. In optical measurements for coarse-grained specimens, the preferred orientation of all of the three crystallographic axes of individual grains was determined with the universal stage. This is not possible to do for fine-grained specimens, so we measured the orientation of the {110} twin boundaries on the polished and etched sections. About 2000 twin boundaries were measured.
25. We used laboratory data on $MgSiO_3$ perovskite at ambient conditions. The effects of pressure and temperature on individual elastic constants in perovskite are not known, and neither are the effects of Fe, which might change the anisotropy. However, in the case of olivine, the temperature and pressure effects and also the effects of Fe on anisotropy are weak (*7*), so therefore we assume that the elastic anisotropy of perovskite in the lower mantle is similar to that measured at room pressure and temperature (*11*).
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27. Shear wave splitting was studied near subduction zones (*9*). Seismic tomography (*15*) and some numerical simulations [P. Tackley, D. J. Stevenson, G. A. Glazmaier, G. Schubert, *Nature* **361**, 699 (1993); S. Honda, D. A. Yuen, S. Balachandar, D. Reuteler, *Science* **259**, 1308 (1993)] suggest that the dominant geometry of deformation in these regions is vertical shear.
28. The seismic anisotropy for samples deformed by superplastic creep was not calculated because the complete crystallographic orientation of individual grains was measured only for coarse-grained samples deformed by dislocation creep. However, the nearly random orientation of grains found by x-ray measurements (Fig. 2) indicates that seismic anisotropy must be very weak.
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Large-Scale Interplanetary Magnetic Field Configuration Revealed by Solar Radio Bursts

M. J. Reiner, J. Fainberg, R. G. Stone

An instantaneous view of the interplanetary extension of the solar magnetic field is provided here by measurements from a space platform at high ecliptic latitudes of the trajectories of individual type III solar radio bursts. The Ulysses spacecraft provides this unique vantage point with an orbit taking it far from the ecliptic plane. The Ulysses radio measurements illustrate the capability of detecting and tracking coronal disturbances as they propagate through the interplanetary medium.

Solar energetic processes, such as solar flares, inject energetic electrons into interplanetary space, where, as a result of their small gyroradii, these electrons are constrained to follow the interplanetary extension of the solar magnetic field. As the suprathermal electrons propagate through the interplanetary medium, they interact with the local plasma to generate radio emissions (*1*) at the plasma frequency and its harmonic (*2*). Because the plasma frequency (proportional to the square root of the solar wind plasma density) falls off with increasing heliocentric distance, radio emission generated farther from the sun occurs at lower frequencies. For radio emission generated from regions near the sun to near Earth's orbit at 1 astronomical unit (1 AU = 1.5×10^8 km), the plasma frequency ranges from several hundreds of megahertz to ~50 kHz; the precise values depend on the interplanetary conditions at the time of the radio emission and on whether the emission occurs at the fundamental or harmonic of the plasma frequency. Both direct observations (*3*, *4*) and modeling (*5*) indicate that the radio emission is broadly beamed in the direction of the interplanetary magnetic field, but the value of the beamwidth is difficult to estimate. The radio manifestation of the interaction of the electrons with the solar wind as they propagate along the magnetic field lines through the interplanetary medium is known as a type III radio burst (*6*). Low-frequency type III radio bursts have been observed for decades by spacecraft located in the ecliptic plane (*7*).

The Ulysses spacecraft, launched in October 1990, used the intense gravitational field of Jupiter to rotate its orbital plane far out of the ecliptic; it became the first spacecraft to go to high ecliptic latitudes and over the poles of the sun (*8*). During the southern polar pass, which occurred during September and October of 1994, Ulysses went as high as 80°S ecliptic latitude at a distance of ~2 AU below the ecliptic plane. After a rapid passage through the ecliptic plane (~1° day^{-1} in ecliptic latitude), Ulysses made a northern polar pass, reaching a maximum northern ecliptic latitude of 79°N in late August 1995. The instrumentation on Ulysses includes a sensitive radio receiver (*9*) with 76 discrete frequency channels covering the range from 1 to 940 kHz. This receiver is coupled to a 72.5-m (tip to tip) dipole antenna in the spin plane of the spacecraft and a 7.5-m monopole antenna along the spacecraft spin axis. During the in-ecliptic phase of the Ulysses mission, thousands of type III radio bursts were observed by this instrument. The Ulysses radio experiment was designed to determine the direction of arrival of the radiation from the measured modulation in the radio signals as the spacecraft spins about its axis (*10*). Ulysses observations therefore permit tracking of type III radio sources through interplanetary space.

During its trajectory to high latitudes, Ulysses continued to observe type III radio emission (*11*). This observation is significant because solar active regions, from

M. J. Reiner, Hughes STX, Lanham, MD 20706, USA.
J. Fainberg and R. G. Stone, NASA Goddard Space Flight Center, Greenbelt, MD 20771, USA.

which the type III–generating suprathermal electrons originate, are generally confined to low solar latitudes [~10° north or south of the solar equator at solar minimum (*12*)]. It is therefore expected that the suprathermal electrons, and hence the type III radio sources, should be confined to relatively low-latitude interplanetary field lines. Indeed, evidence has been presented that most type III radio trajectories lie within 30° of the ecliptic plane (*13*). Because type III radio emission is beamed in the direction of the magnetic field, at sufficiently high latitudes the spacecraft may eventually move out of the beam pattern.

There are two possible reasons why type III radio bursts are observed at high latitudes: the latitudinal beamwidth is very broad or the radiation is scattered to high latitudes. A broad latitudinal beamwidth is suggested in analogy with estimates that the longitudinal beamwidth is greater than 100° (*5*); scattering is suggested since it is known to play an important role because of density inhomogeneities in the interplanetary medium near radio source regions (*4*).

The radio emission generated by the interaction of the suprathermal electrons with the solar wind produces frequency-drifting signatures in the measured dynamic spectrum (Fig. 1A). These are the identifying characteristics of type III radio bursts. During the 12-hour period of Fig. 1, at least four type III radio bursts were detected, with a particularly intense burst occurring at ~10:15 UT that extended from 940 kHz to below 30 kHz. The emission at high frequency (~940 kHz) corresponds to the radio source near (~0.1 AU) the sun; emission at low frequency (~63 kHz) corresponds to the radio source, and therefore the electrons, far (~1 AU) from the sun. Because of the large but finite speed of the exciter electrons [between 0.1*c* and 0.3*c* (*14*), where *c* is the speed of light in a vacuum], the onset of the radio emission occurs at progressively later times at lower frequencies, hence the observed frequency drift. At these speeds, it takes the exciter electrons about 20 min to travel from the sun to 1 AU. The duration of the radio emission increases with decreasing frequency from ~10 min at 940 kHz to ~1.5 hours at 63 kHz.

The type III intensity profiles (Fig. 1B) display no obvious differences from burst profiles previously observed from spacecraft located in the ecliptic plane (*7*); they consist of a rapid rise to a maximum intensity followed by a slower exponential-like decay. Ulysses has observed numerous type III radio bursts like those in Fig. 1 from high ecliptic latitudes.

We measured the direction of arrival of the radiation at each observing frequency for the intense burst in Fig. 1 beginning at 10:15 UT. This burst was associated with a solar flare at ~10:08 UT that occurred in an active region located at 6°S, 11°W on the solar surface as seen from Earth. Making the reasonable assumption that the radio sources originating from this flare site remain close to the ecliptic plane, the intersections of the arrival directions with the ecliptic plane at each frequency (Fig. 2) should approximate the radio trajectory through interplanetary space for this type III burst. The type III radio trajectory which represents the average path followed by the suprathermal exciter electrons follows a spiral-like path corresponding to heliocentric distances from ~0.1 to 0.8 AU, in close approximation to a 400-km s^{-1} Archimedean spiral (solid blue curve). Deviations from the Archimedean spiral path result from temporal variations in the interplanetary plasma, which distort the spiral field lines, and from small errors in the observed radio source directions. Because of the unique perspective of these observations, any inclination of the actual radio trajectory above or below the ecliptic plane would not alter the spiral characteristic; it would imply only a different solar wind speed.

Five days later, at about 13:35 UT on 30 October 1994, Ulysses observed another intense type III radio burst. This burst was not associated with an observed solar flare, but there was an active region on the sun at 18°N, 13°E (relative to Earth) that may have been the origin of the energetic electrons. This radio trajectory also followed a spiral-like path (Fig. 2), this time corresponding to a solar wind speed of ~300 km s^{-1} (dashed blue curve). The radio source locations shown range in frequency from 940 to 81 kHz, corresponding to heliocentric distances from 0.1 to 1.1 AU, suggesting that the interplanetary density along the 30 October trajectory was somewhat higher than that along the 25 October trajectory; this reflects different interplanetary conditions encountered at these two locations and time periods. This burst illustrates the advantage of tracking type III radio bursts far from the ecliptic plane. From the location of Ulysses at high ecliptic latitudes, radio bursts originating from any solar longitude can be clearly viewed and tracked through interplanetary space. By contrast, from the perspective of an observer near

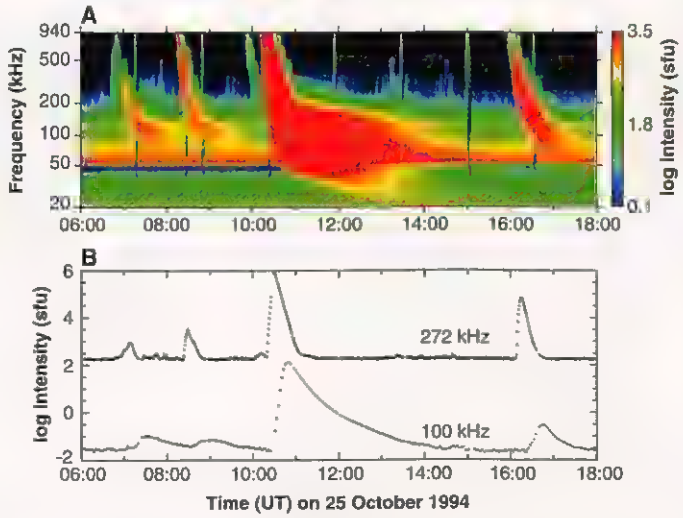

Fig. 1. (**A**) Dynamic spectral representation of the Ulysses radio data from 06:00 to 18:00 UT (universal time at the spacecraft) on 25 October 1994, showing four frequency-drifting type III radio bursts observed by Ulysses from 78°S ecliptic latitude (74°S heliographic latitude). The color scale shows the intensity in solar flux units (sfu) (1 sfu = 10^{-22} W m^{-2} Hz^{-1}). The horizontal band at about 50 kHz is an artifact of the transition between two different radio receivers. (**B**) Intensity versus time plots for two discrete frequency channels showing the profiles of the type III radio bursts in (A). The profile for 100 kHz was downshifted by 4 units for clarity of presentation.

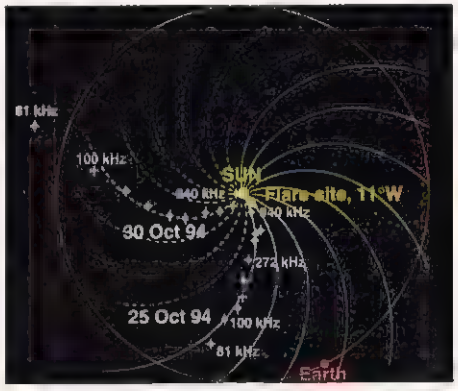

Fig. 2. A view of the ecliptic plane from the northern ecliptic pole, showing measured radio trajectories for two type III radio bursts observed by Ulysses on 25 and 30 October 1994 from ~2 AU above the south solar pole. Both trajectories follow spiral-like paths through interplanetary space from near the sun to ~1 AU. The stars correspond to the radio source locations at 10 discrete observing frequencies extending from 940 to 81 kHz. The blue curves are Archimedean (Parker) spirals corresponding to solar wind speeds of 300 km s^{-1} (dashed curves) and 400 km s^{-1} (solid curves). The flare site for the 25 October event, relative to the corresponding trajectory and to the location of the Earth, is also indicated.

Earth, this burst would be difficult to track because of both the foreshortening of the spiral path and the passage of the burst around the east limb of the sun.

Because the entire radio trajectory, and therefore the underlying magnetic field structure, from the sun to ~1 AU was measured in less than 1 hour, the measured radio trajectories provide an essentially instantaneous visualization of the spiral magnetic field configuration. Figure 2 represents a radio "snapshot" taken by Ulysses from high ecliptic latitudes of conditions in the solar wind near the ecliptic plane during the time of these bursts. From the measured path lengths followed by the exciter electrons along the radio trajectories in Fig. 2 and from the measured onset times of the radio emission at different frequencies determined from the burst profiles (such as shown in Fig. 1B), we find that the average exciter speed for both bursts lies between 0.3c and 0.4c, which is consistent with more energetic exciters previously reported (*14*).

The results in Fig. 2 can be understood in terms of Parker's (*15*) prediction that the interplanetary extension of the solar coronal magnetic field is wound into Archimedean spirals because of the rotation of the sun and the radial outflow of the solar wind. The curvature of the spiral is fixed by the solar wind speed; a slow solar wind speed corresponds to a more tightly wound spiral. The average spiral structure of the coronal magnetic fields has since been confirmed by numerous in situ spacecraft measurements of the average angle of the magnetic field vector (*16*). Such single point measurements, however, do not reveal the instantaneous spiral structure out to 1 AU. The Ulysses radio observations provide a direct unequivocal confirmation of Parker's 1958 prediction of the interplanetary magnetic field topology under quiet solar wind conditions.

Fig. 3. A view of the ecliptic plane from the northern ecliptic pole, showing measured radio trajectories for two type III radio bursts observed by Ulysses on 26 and 27 November 1994 from 68°S ecliptic (62°S heliographic) latitude and ~1.8 AU from the sun. The second trajectory is rotated by ~14.4° to take into account the rotation of the sun during this time interval. The numbers near the points refer to the frequency (in kilohertz) at that point. The radio source locations for the 26 November event are shown at discrete frequencies ranging from 940 to 52 kHz, and for the 27 November event, ranging from 940 to 81 kHz. These trajectories follow paths that deviate significantly from an Archimedean spiral. They may indicate the presence of a transient interplanetary disturbance propagating outward through the interplanetary medium.

Tracking of individual type III radio bursts from spacecraft located in the ecliptic plane was previously used to derive an instantaneous picture of the average spiral magnetic field topology from the sun to 1 AU (*17*). The radio trajectories derived by this method were often difficult to obtain because, in addition to limb effects and the foreshortening of the interplanetary field lines for an observer in the ecliptic plane, it was necessary to assume an average interplanetary density-distance scale. The determination of the radio burst trajectories was very sensitive to the relation between the electron plasma density and radial distance from the sun, which is only an average approximation and does not take into account the large temporal variations in solar wind velocity and density. Nevertheless, Fainberg, Evans, and Stone used IMP-6 (Interplanetary Monitoring Platform) radio data to trace the global spiral magnetic field configuration from the sun to 1 AU (*18*); such observations provided the first indirect observation of the global spiral structure of interplanetary magnetic fields. By contrast, no assumptions had to be made about a density-distance scale in our determination of the radio source locations (*19*).

Another problem affecting observations made by spacecraft near the ecliptic is that scattering and refraction of radio waves by density inhomogeneities and possibly associated anomalous time delays (*4*) can significantly alter derived trajectories and exciter speeds. Because the interplanetary plasma is less dense and more homogeneous at high latitudes (*20*), the Ulysses radio observations are affected much less by propagation anomalies.

From the unique perspective of Ulysses at high latitudes, type III radio trajectories can also be used to determine the magnetic configuration in the disturbed solar wind when field lines deviate significantly from the Parker spiral. The radio trajectory for an intense type III radio burst that occurred at 20:30 UT on 26 November (Fig. 3) deviated significantly from an Archimedean spiral. There was a sudden change in direction of the radio trajectory between 740 and 272 kHz. It may have resulted from a transient disturbance propagating through the interplanetary medium. This interpretation is reinforced when we superpose on this plot a radio trajectory for another very intense radio burst, associated with a flare from the same active region (16°S, 10°W), that occurred on the following day, 27 November, at ~16:25 UT. The shape of this latter radio trajectory is similar to the 26 November trajectory, except that the sudden change in direction is observed at lower frequencies, between 196 and 148 kHz, which corresponds to a heliocentric distance about 0.3 AU farther from the sun than for the 26 November burst. A distance of 0.3 AU in one day corresponds to a structure propagating outward through the interplanetary medium at ~600 km s^{-1}, which is a typical speed for a transient interplanetary disturbance. These observations illustrate the potential for tracking transient interplanetary disturbances with type III radio bursts as tracers of the large-scale configuration of the interplanetary magnetic field.

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18. Other techniques for deriving individual type III radio trajectories from spacecraft observations in the ecliptic plane have been developed [M. J. Reiner and R. G. Stone, *Sol. Phys.* **106**, 397 (1986); *Astron. Astrophys.* **206**, 316 (1988)]. These methods did not require a global density-distance scale, however, other assumpt ons had to be introduced to derive the radio trajectories. For periods of continuous radio em ss on observed during interplanetary type III radio storms, J.-L. Bougeret, J. Fainberg, and R. G. Stone [*Astron. Astrophys.* **141**, 17 (1984); *Science* **222**, 506 (1983)] derived radio trajectories out to ~0.7 AU without assuming a density-distance scale. In this case, the resulting spiral path represents an average of the interplanetary magnetic field configuration over several days. By contrast, tracking individual radio bursts provides a

picture of the field configuration over a much shorter time period (~1 hour).

19. From the measured positions in Fig. 2, a density-distance scale that does not differ significantly from that used earlier (7) can be derived; an approximate r^{-2} falloff (r is heliocentric distance) is indicated. These results reinforce the interpretation that the radiation observed by Ulysses occurs at the harmonic of the plasma frequency.
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Role of Yeast Insulin-Degrading Enzyme Homologs in Propheromone Processing and Bud Site Selection

Neil Adames, Kelly Blundell, Matthew N. Ashby, Charles Boone*

The *Saccharomyces cerevisiae AXL1* gene product Axl1p shares homology with the insulin-degrading enzyme family of endoproteases. Yeast *axl1* mutants showed a defect in a-factor pheromone secretion, and a probable site of processing by Axl1p was identified within the a-factor precursor. In addition, Axl1p appears to function as a morphogenetic determinant for axial bud site selection. Amino acid substitutions within the presumptive active site of Axl1p caused defects in propheromone processing but failed to perturb bud site selection. Thus, Axl1p has been shown to participate in the dual regulation of distinct signaling pathways, and a member of the insulinase family has been implicated in propeptide processing.

Peptide hormones secreted by higher eukaryotes are synthesized as larger precursors and released in mature form by the action of specific processing proteases (*1*). Analogous proteolytic maturation occurs for *Saccharomyces cerevisiae* pheromones involved in the mating response of haploid a and α cells (*2*). The pheromone produced by a cells, a-factor, is one of a growing number of secreted proteins, such as interleukin-1α (IL-1α), IL-1β, and some fibroblast growth factors (such as FGF-1 and FGF-2) (*3*), that are processed from precursors before export through a nonclassical secretory pathway. The proteolytic maturation of progenitor a-factor (pro–a-factor) is not well understood and provides an opportunity to identify novel eukaryotic processing enzymes. Here we present genetic evidence implicating yeast homologs of human insulin-degrading enzyme (hIDE), encoded by *AXL1* and *STE23*, in the specific processing of pro–a-factor.

Pro–a-factor is encoded by two genes, *MFA1* and *MFA2*, with products that are 36 and 38 amino acids in length, respectively (*4*, *5*). These precursors contain a single copy of the mature a-factor peptide, with an NH_2-terminal extension and a COOH-terminal CAAX consensus sequence (C is cysteine, A is usually aliphatic, and X can be various amino acids). The cysteine residue of CAAX proteins is isoprenylated within the cytoplasm; such lipid-modified proteins become localized in the membrane, then undergo endoproteolysis of the three terminal AAX residues and methylesterification of the free carboxylate group on the prenylated cysteine (*6*). The genes encoding most of the enzymes responsible for these modifications have been characterized (*7*, *8*), and mutations in these genes abolish a-factor secretion, leading to an a-specific mating defect (*7*, *8*). After COOH-terminal processing, the a-factor precursor undergoes two sequential NH_2-terminal endoproteolytic events (*8*, *9*). The final NH_2-terminal cleavage generates mature a-factor, a 12–amino acid lipopeptide (*10*, *11*). Ultimately, the *STE6* product, an adenosine triphosphate–hydrolyzing transport protein related to the mammalian multidrug resistance protein (Mdr1), mediates a-factor transport across the cell surface (*12*).

To identify genes required for pro–a-factor maturation, we mutagenized a cells and screened for strains that showed a reduced mating efficiency as well as a defect in secreted a-factor activity (*13*). A mutant allele, designated *ste22-1*, identified a novel gene required for normal amounts of secreted a-factor activity (Fig. 1A). In crosses to a wild-type α strain, we found that the sterility cosegregated with this reduced pheromone production and that α *ste22-1* cells mated with normal efficiency (Fig. 1A). A similar but more severe a-specific phenotype was associated with a strain from which the a-factor structural genes *MFA1* and *MFA2* were deleted (Fig. 1A). Thus, *ste22-1* mutants exhibited an a-specific mating defect that appeared to be caused by reduced secretion of active pheromone.

We cloned *STE22* by complementation of the mating defect of *ste22-1* cells, using a yeast genomic library. Four plasmids, with overlapping genomic inserts, that complemented both the sterility and the reduced a-factor production were isolated. Subcloning and sequence analysis revealed that *STE22* was identical to *AXL1* (*14*). Thus, *AXL1* rescued the mating defect of *ste22-1* cells (Fig. 1B). The *AXL1* product is required for generation of the axial budding pattern displayed by haploid cells (*14*) and shares sequence similarity with members of a family of endoproteases whose archetypes are hIDE and *Escherichia coli* protease III (*15*)–metalloproteases with a preference for small peptide substrates (*16*, *17*). In vivo, hIDE is implicated in the degradation of intracellular insulin, whereas the physiological substrate of protease III remains unknown (*16*, *17*). Another member of the hIDE family, rat *N*-arginine dibasic convertase (NRDC), is proposed to function as a prohormone processing enzyme (*18*). A highly conserved domain is present in hIDE-like sequences that is likely to be important both for proteolysis and for metal binding (Fig. 2A) (*17*).

An *axl1::URA3* disruption was constructed (Fig. 2B) and introduced into a diploid strain that was heterozygous for *ste22-1*. Sporulation and tetrad analysis revealed that *axl1::URA3* was tightly linked to *ste22-1* (*19*). Moreover, the *axl1::URA3* phenotype was comparable to that of *ste22-1* (Fig. 1A). These data confirmed that *ste22-1* was a mutant allele of *AXL1*. Mutations in *AXL1* cause haploid cells to bud with a bipolar pattern that is normally displayed by diploid cells (*14*). Because the genetic background of the strain we used for the mutant isolation is defective for bud site selection (*20*), we constructed an *axl1* disruption in a haploid strain normal for axial budding, EG123. This *axl1::URA3* mutant showed both a bipolar budding pattern and a mating defect associated with reduced a-factor secretion (*19*).

Because insulinase homologs can function as specific endoproteases (*18*), Axl1p may act as a propheromone processing enzyme. We tested an *axl1* mutant for defects in COOH-terminal CAAX-proteolysis and found it to be unaffected (*21*). To address the possibility that Axl1p is involved in NH_2-terminal processing, a-factor proteins were labeled with [^{35}S]cysteine in a pulse-chase protocol, then immunoprecipitated from both intracellular and extracellular fractions and subjected to polyacrylamide gel electrophoresis (PAGE). Three different intracellular a-factor peptides were observed in cells containing a functional

N. Adames, K. Blundell, C. Boone, Institute of Molecular Biology and Biochemistry, Simon Fraser University, Burnaby, British Columbia, V5A 1S6, Canada.
M. N. Ashby, Department of Molecular and Cell Biology, University of California, Berkeley, CA 94720, USA.
*To whom correspondence should be addressed.

AXL1 gene (Fig. 2C). The largest form, designated P1, has been assigned to a propheromone molecule with a completely modified COOH-terminus, yet retains an unprocessed NH_2-terminal extension (Fig. 2D) (*8*, *9*). The P2 propheromone is derived from P1 through proteolytic cleavage within the NH_2-terminal domain of the precursor (*8*, *9*). The species designated M corresponds to mature a-factor, which results from a second cleavage event within the NH_2-terminal region of P2 (*8*, *9*). Only the mature pheromone is efficiently exported across the plasma membrane and recovered from the extracellular fraction (*8*, *9*). In *axl1::URA3* cells, the P1 propheromone was processed to the P2 form at a normal rate, but the P2 intermediate was processed inefficiently and remained relatively stable for the duration of the chase period (Fig. 2C). Consequently, *axl1::URA3* mutants produced small amounts of exported pheromone (Fig. 2C). Thus, the sterility of *axl1* cells largely resulted from a defect in P2 propheromone processing and reduced secretion of mature a-factor.

Although propheromone processing was greatly impaired, *axl1* mutants were able to secrete residual levels of bioactive a-factor (Fig. 1), which implied that *S. cerevisiae* contains a functional homolog of Axl1p. The chromosome XII sequencing project has identified a sequence, L8084.12 (*22*), which we call *STE23*, encoding a new member of the insulin-degrading enzyme family (Fig. 2A). To assess the function of *STE23*, we created a *ste23Δ::URA3* deletion mutation (*23*). When introduced into strains with a wild-type *AXL1* gene, the *ste23Δ::URA3* mutation failed to perturb mating efficiency or secreted a-factor activity (Fig. 1C). In contrast, experiments with the *axl1::URA3 ste23Δ::URA3* double mutant clearly showed that *ste23Δ::URA3* accentuated both the mating and secreted-pheromone defects of *axl1::URA3* cells (Fig. 1C). Pulse-chase studies indicated that the a-factor precursor was processed normally in *ste23Δ::URA3* cells; however, as observed for *axl1::URA3* cells, the *axl1::URA3 ste23Δ::URA3* double mutant accumulated the P2 propheromone intracellularly (*19*). Thus, our findings are consistent with the possibility that Ste23p is responsible for the residual propheromone processing displayed by *axl1* mutants. We also examined the effect of *ste23Δ::URA3* on bud site selection by means of a microcolony assay and calcofluor staining of bud scars (*24*). EG123 *ste23Δ::URA3* cells bud with an axial pattern, and the *axl1::URA3 ste23Δ::URA3* double mutant generates the bipolar pattern also observed for *axl1::URA3* cells (*19*). Therefore, Ste23p does not appear to participate in bud site selection.

Both hIDE and protease III contain a sequence motif with two invariant histidine residues and a glutamate, HXXEH (*17*). Substitutions for either of the histidine residues cause a loss of proteolytic activity as well as a defect in metal binding (*17*). The glutamate residue is also important for proteolysis, and by analogy with the function ascribed to a similar motif found in thermolysin, the carboxylate side chain of the glutamate is expected to participate in catalysis (*25*). By site-specific mutagenesis, we generated three variants of the HXXEH sequence found within Axl1p (Fig. 2A) that correspond to substitutions of the first histidine (*axl1-H68A*), and the glutamate residue (*axl1-*

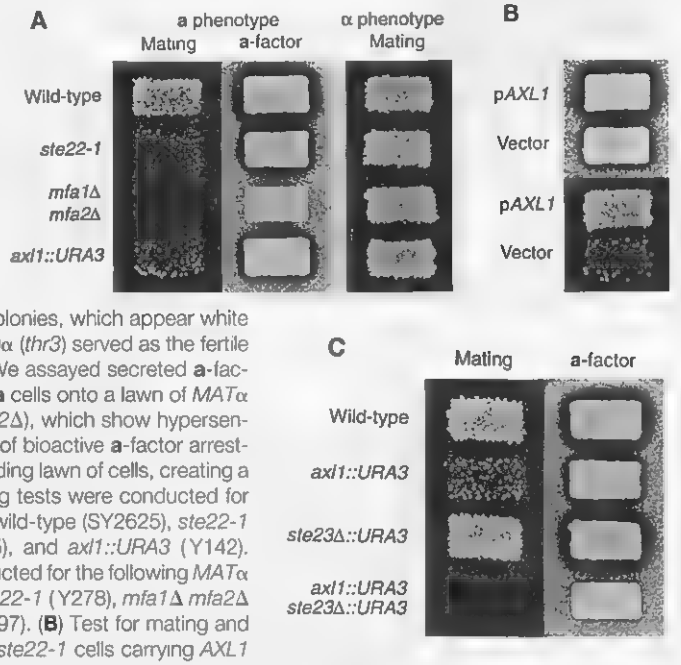

Fig. 1. Mating efficiency and secreted **a**-factor activity associated with *ste22* (*axl1*) mutants. Mating and secreted **a**-factor assays were determined for a set of coisogenic strains that differed only at the indicated loci (*30*) as described previously (*28*). The mating efficiency is reflected by the formation of diploid colonies, which appear white on the black background. 70α (*thr3*) served as the fertile *MATα* mating tester strain. We assayed secreted **a**-factor activity by patching *MAT***a** cells onto a lawn of *MATα* cells, SY2014 (*ste3Δ306 sst2Δ*), which show hypersensitivity to **a**-factor. Secretion of bioactive **a**-factor arrested the growth of the surrounding lawn of cells, creating a clear zone or halo. (**A**) Mating tests were conducted for the following *MAT***a** strains: wild-type (SY2625), *ste22-1* (Y49), *mfa1Δ mfa2Δ* (Y115), and *axl1::URA3* (Y142). Mating tests were also conducted for the following *MATα* strains: wild-type (Y199), *ste22-1* (Y278), *mfa1Δ mfa2Δ* (Y195), and *axl1::URA3* (Y197). (**B**) Test for mating and secreted **a**-factor activity of *ste22-1* cells carrying *AXL1* (p*AXL1*) or the vector (pRS316) (*23*). (**C**) Mating efficiency and secreted **a**-factor activity associated with *ste23* mutants. Mating and **a**-factor assays were conducted for the following *MAT***a** strains: wild-type (SY2625), *axl1::URA3* (Y142), *ste23Δ::URA3* (Y221), and the *axl1::URA3 ste23Δ::URA3* double mutant (Y220) (*30*).

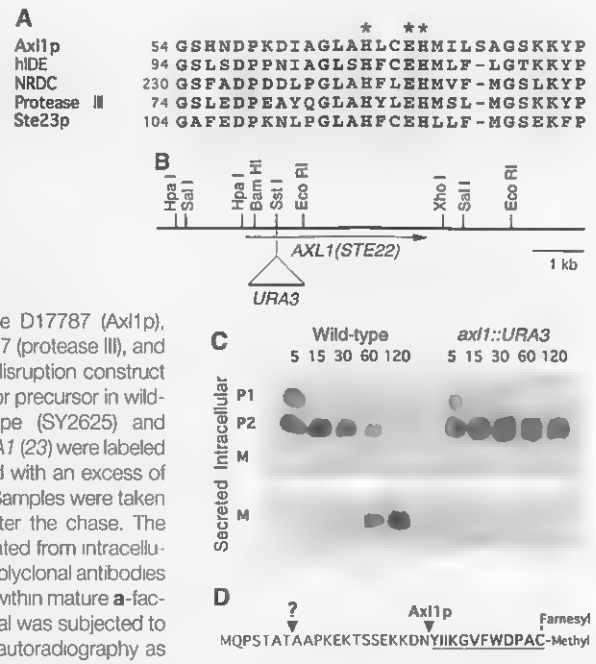

Fig. 2. *AXL1* disruption construct and an alignment of amino acid sequences from within the proposed active site domain of the insulin-degrading enzyme family of metalloendoproteases. (**A**) Alignment of a highly conserved sequence found within the insulin-degrading enzyme family of endoproteases (*29*). Asterisks mark key residues of the HXXEH motif that are required for proteolysis by hIDE and protease III (*17*). GenBank accession numbers for these sequences are D17787 (Axl1p), M21188 (hIDE), L27124 (NRDC), X06227 (protease III), and U19729 (Ste23p). (**B**) The *axl1::URA3* disruption construct (*23*). (**C**) Processing of the *MFA1* **a**-factor precursor in wild-type and *axl1* mutant cells. Wild-type (SY2625) and *axl1::URA3* cells (*30*) containing YEp*MFA1* (*23*) were labeled with [^{35}S]cysteine for 5 min and chased with an excess of unlabeled cysteine and methionine (*8*). Samples were taken at the indicated number of minutes after the chase. The **a**-factor peptides were immunoprecipitated from intracellular and secreted fractions by means of polyclonal antibodies that were specific for the peptide found within mature **a**-factor (*11*). The immunoprecipitated material was subjected to SDS-PAGE analysis and visualized by autoradiography as described previously (*8*). P1 designates the **a**-factor precursor with a fully modified COOH-terminus, P2 designates the precursor with the first seven amino acids removed (*8*, *9*), and M designates mature **a**-factor. (**D**) The structure of the **a**-factor propheromone P1. Arrowheads indicate the proposed positions of cleavages that would generate P2 (*9*) and mature **a** factor (underlined). The latter cleavage is *AXL1*-dependent.

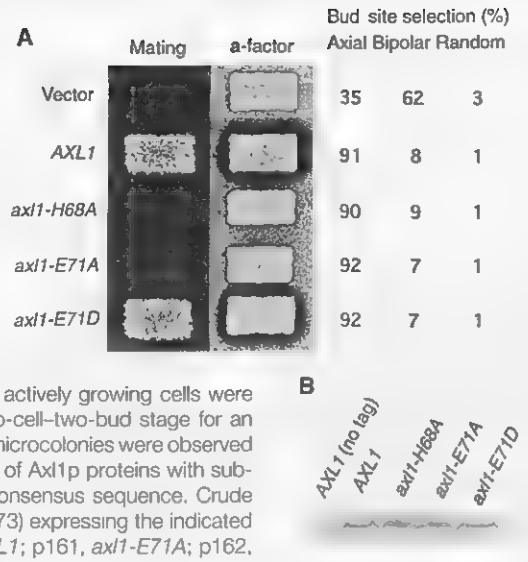

Fig. 3. (**A**) Mating efficiency, secreted **a**-factor activity, and bud site selection were determined for *axl1Δ::LEU2 ste23Δ::LEU2* double mutant cells carrying substitutions that alter the proposed active site of Axl1p. Cells were transformed with HA epitope–tagged versions of *AXL1*, *axl1-H68A*, *axl1-E71A*, and *axl1-E71D* (p151, p162, p161, and p163, respectively), or with the vector (YEp352) (*31*). Mating and secreted pheromone assays (*28*) were conducted with Y231 transformants, and bud site selection assays (*24*) were conducted with Y272 transformants (*30*). Before these assays, the cells were grown on selective medium to maintain plasmids. For bud site selection, actively growing cells were spread on agar slabs, and scored at the two-cell–two-bud stage for an axial, bipolar, or random pattern. At least 600 microcolonies were observed for each transformant. (**B**) Steady-state levels of Axl1p proteins with substitutions in the metalloprotease active site consensus sequence. Crude extracts prepared from *axl1Δ::LEU2* cells (Y173) expressing the indicated *AXL1* alleles, either with an HA tag (p151, *AXL1*; p161, *axl1-E71A*; p162, *axl1-H68A*; p163, *axl1-E71D*) or without an HA tag (p129, no tag) (*31*) were analyzed by immunoblotting with 12CA5 antibodies (Boehringer Mannheim).

E71A and *axl1-E71D*). Both the *axl1-H68A* and *axl1-E71A* alleles failed to restore pheromone production and mating to an *axl1 ste23* double mutant, whereas the more conservative substitution associated with *axl1-E71D* complemented these mutant phenotypes (Fig. 3A). The loss of function associated with *axl1-H68A* and *axl1-E71A* was not due to reduced product expression or stability, because epitope-tagged versions of the altered proteins were detected at steady-state levels that were identical to that of the wild-type protein (Fig. 3B). It is likely that Axl1p functions as a metalloprotease involved in propheromone processing with the participation of His^{68} and Glu^{71} in a thermolysin-like mode of proteolysis.

On the basis of the homology shared with hIDE, it has been suggested that Axl1p contributes to bud site selection through a proteolytic mechanism (*14*). As a test of this hypothesis, we examined the budding pattern resulting from expression of *axl1* alleles altered in the metalloprotease active site consensus sequence. Although defective for a-factor secretion and mating, we found that cells expressing *axl1-H68A* and *axl1-E71A* budded with a normal axial pattern (Fig. 3A). Thus, these mutations genetically separated the pheromone processing and bud site selection functions associated with *AXL1*, which suggests that participation of Axl1p in axial budding does not require proteolysis.

We have demonstrated a processing phenotype for *axl1* mutants that is consistent with Axl1p acting as a specific endoprotease involved in pro–**a**-factor maturation. These results combine with those of Fujita *et al.* (*14*) to implicate *AXL1* in the dual regulation of intercellular signaling during the yeast mating response and intracellular signaling for axial bud site selection. Our findings imply that the proteolytic activity of Axl1p is required to process a farnesylated and membrane-bound form of pro–a-factor (*8*) to generate mature pheromone for secretion by Ste6p (*26*); therefore, we suggest that Axl1p may localize to the inner surface of the plasma membrane. Surface localization would also allow Axl1p to contribute to bud site selection through interaction with the products of other genes required for axial budding, including Bud3p and Bud4p, or components of the Rsr1p (Bud1p) Ras-like guanosine triphosphatase cycle (*27*). These interactions could involve proteolysis, but our results suggest that Axl1p contributes to axial budding through a nonproteolytic mechanism. Regardless of the role for Axl1p in bud site selection, its requirement for pro–a-factor maturation supports the notion that insulinase homologs function as eukaryotic propeptide convertases (*18*).

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13. SY2625 (*MATa ura3-1 leu2-3, 112 trp1-1 ade2-1 can1-100 sst1Δ mfa2Δ::FUS1-lacZ his3Δ::FUS1-HIS3*) cells were plated onto minimal medium that lacked histidine and contained 0.01 ng of synthetic α-factor per milliliter (Sigma), then mutagenized to 10% survival by exposure to ultraviolet light and allowed to form colonies. This amount of synthetic α-factor does not cause G_1 arrest but leads to induced expression of the *FUS1-HIS3* construct, and thereby allows growth of SY2625 on medium lacking histidine. This regime precluded the isolation of mutations that cause reduced signaling of the pheromone response pathway, because such mutations will lead to a slower growth rate on medium lacking histidine. To identify sterile mutants, the mutagenized cells were scored for the ability to mate to 70α (*thr3*) with the use of a replica-plating procedure (*28*). Ten mutants that were defective in secreted **a**-factor activity (*28*) were isolated and transformed with plasmids carrying the genes previously known to be involved in pro–**a**-factor processing. The defects in four mutants were complemented by *STE6*, four were complemented by *RAM1*, and one was complemented by *STE14* .
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20. F. Cvrckova and K. Nasmyth, *EMBO J.* **12**, 5277 (1993).
21. Ras and **a**-factor converting enzyme (RACE) assays were done by quantification of the release of the COOH-terminal tritiated tripeptide from the **a**-factor–derived peptide KWDPAC(*S*-trans,trans-farnesyl)V[4,5-^{3}H]IA (*29*) as described [M. N. Ashby and J. Rine, *Methods Enzymol.* **250**, 235 (1995)]. Protease determinations were made both in 3000*g* supernatants (S3 0) and 100,000*g* pellets (P100) in both SY2625 (*AXL1*) and Y49 (*ste22-1*) (*30*). The *ste22-1* mutation did not substantially affect RACE activity in either cell-free extract. Specific activities were 151 versus 151 pmol/min per milligram for the S3.0 extracts and 250 versus 293 pmol/min per milligram for the P100 extract in SY2625 and Y49, respectively.
22. M. Johnston *et al.*, the complete sequence of *Saccharomyces cerevisiae* chromosome XII (in preparation).
23. p*AXL1* is a pRS316 plasmid [R. S. Sikorsk and P. Hieter, *Genetics* **122**, 19 (1989)] containing an 8.5-kb *AXL1* (*STE22*) genomic fragment, isolated from a yeast genomic library by complementation of the *ste22-1* mating defect [C. Boone, K. L. Clark, G. F. Sprague Jr., *Nucleic Acids Res.* **20**, 4661 (1992)]. Plasmid pSM86 contains *mfa1Δ::LEU2* (*5*). To create the *axl1::URA3* mutation contained on p98, a *URA3* fragment was inserted at the Sst I site, which occurs within a 1-kb Eco RI–Bam HI fragment of *AXL1*, carried on pUC19. The *axl1::URA3* mutation alters the

Axl1p sequence following Ser^{206} and occurs within the domain of Axl1p that shows homology with hIDE (*14*). To delete the complete *STE23* sequence and create the *ste23Δ::URA3* mutation, polymerase chain reaction (PCR) primers (5′-TCGGAAGACCTCAT-TCTTGCTCATTTTGATATTGCTC- TGTAGATTG-TACTGAGAGTGCAC-3′; and 5′-GCTACAAACAGC-GTCGACTTGAATGCCCCGACATCTTCGACTGT-GCGGTATTTCACACCG-3′) were used to amplify the *URA3* sequence of pRS316, and the reaction product was transformed into yeast for one-step gene replacement [R. Rothstein, *Methods Enzymol.* **194**, 281 (1991)]. To create the *axl1Δ::LEU2* mutation contained on p114, a 5.0-kb Sal I fragment from p*AXL1* was cloned into pUC19, and an internal 4.0-kb Hpa I–Xho I fragment was replaced with a *LEU2* fragment. To construct the *ste23Δ::LEU2* allele (a deletion corresponding to 931 amino acids) carried on p153, a *LEU2* fragment was used to replace the 2.8-kb Pml I–Ecl136 II fragment of *STE23*, which occurs within a 6.2-kb Hind III–Bgl II genomic fragment carried on pSP72 (Promega). To create YEp*MFA1*, a 1.6-kb Bam HI fragment containing *MFA1*, from pKK16 [K. Kuchler, R. E. Sterne, J. Thorner, *EMBO J.* **8**, 3973 (1989)], was ligated into the Bam HI site of YEp351 [J. E. Hill, A. M. Myers, T. J. Koerner, A. Tzagoloff, *Yeast* **2**, 163 (1986)].

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29. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
30. A W303 1A derivative, SY2625 (*MATa ura3-1 leu2-3, 112 trp1-1 ade2-1 can1-100 sst1Δ mfa2Δ::FUS1-lacZ his3Δ::FUS1-HIS3*), was the parent strain for the mutant search. SY2625 derivatives for the mating assays, secreted pheromone assays, and the pulse-chase experiments included the following strains: Y49 (*ste22-1*), Y115 (*mfa1Δ::LEU2*), Y142 (*axl1::URA3*), Y173 (*axl1Δ::LEU2*), Y220 (*axl1::URA3 ste23Δ::URA3*), Y221 (*ste23Δ::URA3*), Y231 (*axl1Δ::LEU2 ste23Δ::LEU2*), and Y233 (*ste23Δ::LEU2*). *MATα* derivatives of SY2625 included the following strains: Y199 (SY2625 made *MATα*), Y278 (*ste22-1*), Y195 (*mfa1Δ::LEU2*), Y196 (*axl1Δ::LEU2*), and Y197 (*axl1::URA3*). The EG123 (*MATa leu2 ura3 trp1 can1 his4*) genetic background was used to create a set of strains for analysis of bud site selection. EG123 derivatives included the following strains: Y175 (*axl1Δ::LEU2*), Y223 (*axl1::URA3*), Y234 (*ste23Δ::LEU2*), and Y272 (*axl1Δ::LEU2 ste23Δ::LEU2*). *MATα* derivatives of EG123 included the following strains: Y214 (EG123 made *MATα*) and Y293 (*axl1Δ::LEU2*). All strains were generated by means of standard genetic or molecular methods involving the appropriate constructs (*23*). In particular, the *axl1 ste23* double mutant strains were created by crossing of the appropriate *MATa ste23* and *MATα axl1* mutants, followed by sporulation of the resultant diploid and isolation of the double mutant from nonparental di-type tetrads. Gene disruptions were confirmed with either PCR or Southern (DNA) analysis.
31. p129 is a YEp352 [J. E. Hill, A. M. Myers, T. J. Koerner, A. Tzagoloff, *Yeast* **2**, 163 (1986)] plasmid containing a 5.5-kb Sal I fragment of p*AXL1*. p151 was derived from p129 by insertion of a linker at the Bgl II site within *AXL1*, which led to an in-frame insertion of the hemagglutinin (HA) epitope (DQYPYDVPDYA) (*29*) between amino acids 854 and 855 of the *AXL1* product. pC225 is a KS+ (Stratagene) plasmid containing a 0.5-kb Bam HI–Sst I fragment from p*AXL1*. Substitution mutations of the proposed active site of Axl1p were created with the use of pC225 and site-specific mutagenesis involving appropriate synthetic oligonucleotides (*axl1-H68A*, 5′-GTGCTCACAAAGCGCT-GCCAAACCGGC-3′; *axl1-E71A*, 5′-AAGAATCAT-GTGCGCACAAAGGTGCGC-3′; and *axl1-E71D*, 5′-AAGAATCATGTGATCACAAAGGTGCGC-3′). The mutations were confirmed by sequence analysis. After mutagenesis, the 0.4-kb Bam HI–Msc I fragment from the mutagenized pC225 plasmids was transferred into p*AXL1* to create a set of pRS316 plasmids carrying different *AXL1* alleles, p124 (*axl1-H68A*), p130 (*axl1-E71A*), and p132 (*axl1-E71D*). Similarly, a set of HA-tagged alleles carried on YEp352 were created after replacement of the p151 Bam HI–Msc I fragment, to generate p161 (*axl1-E71A*), p162 (*axl1-H68A*), and p163 (*axl1-E71D*).
32. We thank J. Becker and S. Michaelis for providing a-factor antibodies; S. Michaelis for discussing unpublished results and helping with the pulse-chase experiments; J. Brown, J. Chant, and S. Sanders for their input concerning bud site selection experiments; M. Raymond, F. Taminoi, and M. Whiteway for plasmids; M. Marra for providing the *STE23* genomic fragment; and H. Bussey, J. Brown, N. Davis, T. Favero, C. de Hoog, and S. Kim for comments on the manuscript. Supported by a grant to C.B. from the Natural Sciences and Engineering Research Council of Canada. Support for M.N.A. was from a California Tobacco-Related Disease Research Program postdoctoral fellowship (4FT-0083).

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Quantitative Monitoring of Gene Expression Patterns with a Complementary DNA Microarray

Mark Schena,* Dari Shalon,*† Ronald W. Davis, Patrick O. Brown‡

A high-capacity system was developed to monitor the expression of many genes in parallel. Microarrays prepared by high-speed robotic printing of complementary DNAs on glass were used for quantitative expression measurements of the corresponding genes. Because of the small format and high density of the arrays, hybridization volumes of 2 microliters could be used that enabled detection of rare transcripts in probe mixtures derived from 2 micrograms of total cellular messenger RNA. Differential expression measurements of 45 *Arabidopsis* genes were made by means of simultaneous, two-color fluorescence hybridization.

The temporal, developmental, topographical, histological, and physiological patterns in which a gene is expressed provide clues to its biological role. The large and expanding database of complementary DNA (cDNA) sequences from many organisms (*1*) presents the opportunity of defining these patterns at the level of the whole genome.

For these studies, we used the small flowering plant *Arabidopsis thaliana* as a model organism. *Arabidopsis* possesses many advantages for gene expression analysis, including the fact that it has the smallest genome of any higher eukaryote examined to date (*2*). Forty-five cloned *Arabidopsis* cDNAs (Table 1), including 14 complete sequences and 31 expressed sequence tags (ESTs), were used as gene-specific targets. We obtained the ESTs by selecting cDNA clones at random from an *Arabidopsis* cDNA library. Sequence analysis revealed that 28 of the 31 ESTs matched sequences in the database (Table 1). Three additional cDNAs from other organisms served as controls in the experiments.

The 48 cDNAs, averaging ~1.0 kb, were amplified with the polymerase chain reaction (PCR) and deposited into individual wells of a 96-well microtiter plate. Each sample was duplicated in two adjacent wells to allow the reproducibility of the arraying and hybridization process to be tested. Samples from the microtiter plate were printed onto glass microscope slides in an area measuring 3.5 mm by 5.5 mm with the use of a high-speed arraying machine (*3*). The arrays were processed by chemical and heat treatment to attach the DNA sequences to the glass surface and denature them (*3*). Three arrays, printed in a single lot, were used for the experiments here. A single microtiter plate of PCR products provides sufficient material to print at least 500 arrays.

Fluorescent probes were prepared from total *Arabidopsis* mRNA (*4*) by a single round of reverse transcription (*5*). The *Arabidopsis* mRNA was supplemented with human acetylcholine receptor (AChR) mRNA at a dilution of 1:10,000 (w/w) before cDNA synthesis, to provide an internal standard for calibration (*5*). The resulting fluorescently labeled cDNA mixture was hybridized to an array at high stringency (*6*) and scanned

M. Schena and R. W. Davis, Department of Biochemistry, Beckman Center, Stanford University Medical Center, Stanford, CA 94305, USA.
D. Shalon and P. O. Brown, Department of Biochemistry and Howard Hughes Medical Institute, Beckman Center, Stanford University Medical Center, Stanford, CA 94305, USA.

*These authors contributed equally to this work.
†Present address: Synteni, Palo Alto, CA 94303, USA.
‡To whom correspondence should be addressed. E-mail: pbrown@cmgm.stanford.edu

with a laser (3). A high-sensitivity scan gave signals that saturated the detector at nearly all of the *Arabidopsis* target sites (Fig. 1A). Calibration relative to the AChR mRNA standard (Fig. 1A) established a sensitivity limit of ~1:50,000. No detectable hybridization was observed to either the rat glucocorticoid receptor (Fig. 1A) or the yeast *TRP4* (Fig. 1A) targets even at the highest scanning sensitivity. A moderate-sensitivity scan of the same array allowed linear detection of the more abundant transcripts (Fig. 1B). Quantitation of both scans revealed a range of expression levels spanning three orders of magnitude for the 45 genes tested (Table 2). RNA blots (7) for several genes (Fig. 2) corroborated the expression levels measured with the microarray to within a factor of 5 (Table 2).

Differential gene expression was investigated with a simultaneous, two-color hybridization scheme, which served to minimize experimental variation inherent in the comparison of independent hybridizations. Fluorescent probes were prepared from two mRNA sources with the use of reverse transcriptase in the presence of fluorescein- and lissamine-labeled nucleotide analogs, respectively (5). The two probes were then mixed together in equal proportions, hybridized to a single array, and scanned separately for fluorescein and lissamine emission after independent excitation of the two fluorophores (3).

To test whether overexpression of a single gene could be detected in a pool of total *Arabidopsis* mRNA, we used a microarray to analyze a transgenic line overexpressing the single transcription factor *HAT4* (8). Fluorescent probes representing mRNA from wild-type and *HAT4*-transgenic plants were labeled with fluorescein and lissamine, respectively; the two probes were then mixed and hybridized to a single array. An intense hybridization signal was observed at the position of the *HAT4* cDNA in the lissamine-specific scan (Fig. 1D), but not in the fluorescein-specific scan of the same array (Fig. 1C). Calibration with AChR mRNA added to the fluorescein and lissamine cDNA synthesis reactions at dilutions of 1:10,000 (Fig. 1C) and 1:100 (Fig. 1D), respectively, revealed a 50-fold elevation of *HAT4* mRNA in the transgenic line relative to its abundance in wild-type plants (Table 2). This magnitude of *HAT4* overexpression matched that inferred from the Northern (RNA) analysis within a factor of 2 (Fig. 2 and Table 2). Expression of all the other genes monitored on the array differed by less than a factor of 5 between *HAT4*-transgenic and wild-type plants (Fig 1, C

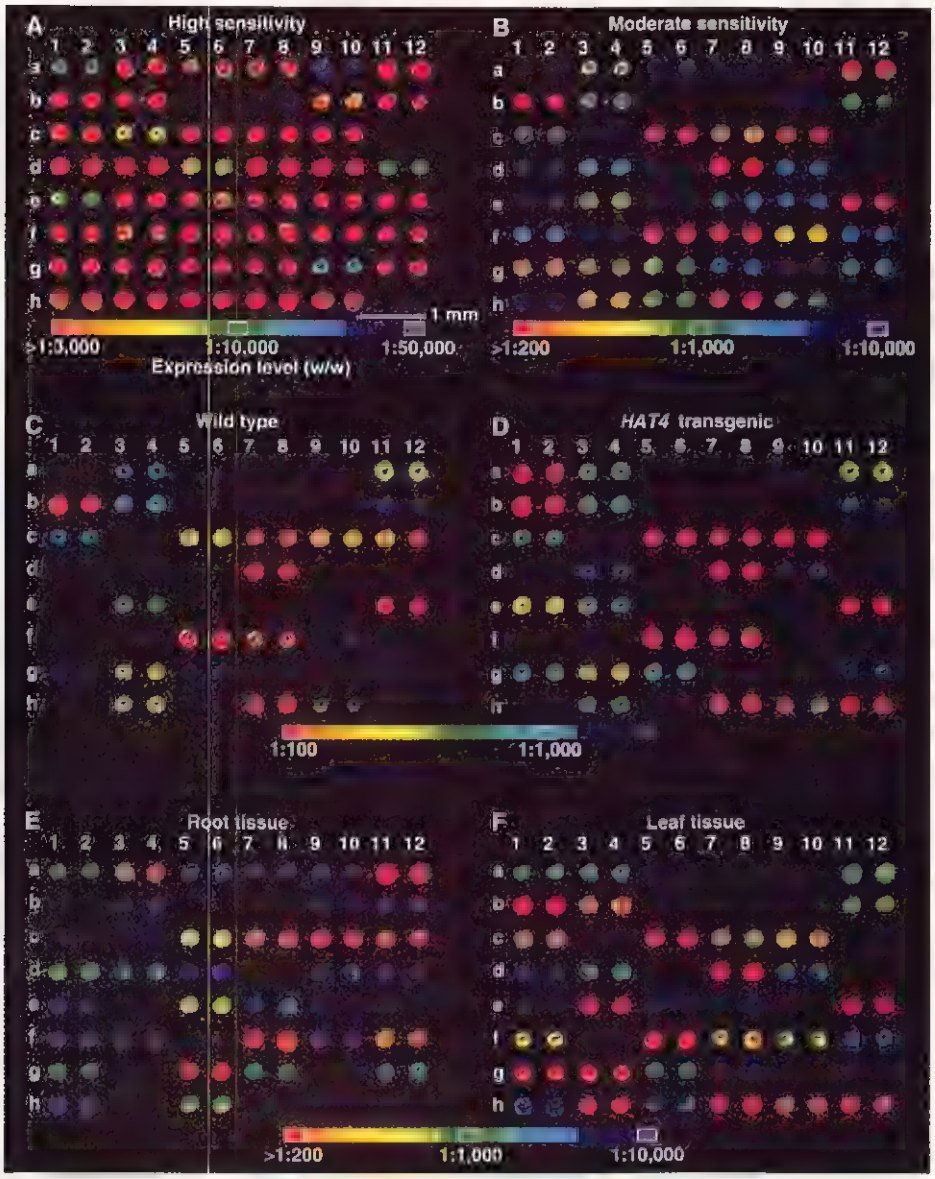

Fig. 1. Gene expression monitored with the use of cDNA microarrays. Fluorescent scans represented in pseudocolor correspond to hybridization intensities. Color bars were calibrated from the signal obtained with the use of known concentrations of human AChR mRNA in independent experiments. Numbers and letters on the axes mark the position of each cDNA. (**A**) High-sensitivity fluorescein scan after hybridization with fluorescein-labeled cDNA derived from wild-type plants. (**B**) Same array as in (A) but scanned at moderate sensitivity. (**C** and **D**) A single array was probed with a 1:1 mixture of fluorescein-labeled cDNA from wild-type plants and lissamine-labeled cDNA from HAT4-transgenic plants. The single array was then scanned successively to detect the fluorescein fluorescence corresponding to mRNA from wild-type plants (C) and the lissamine fluorescence corresponding to mRNA from HAT4-transgenic plants (D). (**E** and **F**) A single array was probed with a 1:1 mixture of fluorescein-labeled cDNA from root tissue and lissamine-labeled cDNA from leaf tissue. The single array was then scanned successively to detect the fluorescein fluorescence corresponding to mRNAs expressed in roots (E) and the lissamine fluorescence corresponding to mRNAs expressed in leaves (F).

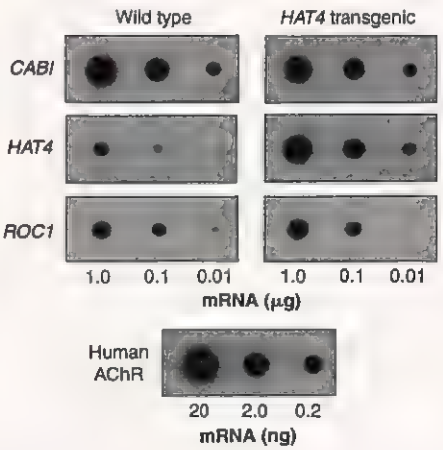

Fig. 2. Gene expression monitored with RNA (Northern) blot analysis. Designated amounts of mRNA from wild-type and *HAT4*-transgenic plants were spotted onto nylon membranes and probed with the cDNAs indicated. Purified human AChR mRNA was used for calibration.

and D, and Table 2). Hybridization of fluorescein-labeled glucocorticoid receptor cDNA (Fig. 1C) and lissamine-labeled *TRP4* cDNA (Fig. 1D) verified the presence of the negative control targets and the lack of optical cross talk between the two fluorophores.

To explore a more complex alteration in expression patterns, we performed a second two-color hybridization experiment with fluorescein- and lissamine-labeled probes prepared from root and leaf mRNA, respectively. The scanning sensitivities for the two fluorophores were normalized by matching the signals resulting from AChR mRNA, which was added to both cDNA synthesis reactions at a dilution of 1:1000 (Fig. 1, E and F). A comparison of the scans revealed widespread differences in gene expression between root and leaf tissue (Fig. 1, E and F). The mRNA from the light-regulated *CAB1* gene was ~500-fold more abundant in leaf (Fig. 1F) than in root tissue (Fig. 1E). The expression of 26 other genes differed between root and leaf tissue by more than a factor of 5 (Fig. 1, E and F).

The *HAT4*-transgenic line we examined has elongated hypocotyls, early flowering, poor germination, and altered pigmentation (*8*). Although changes in expression were observed for *HAT4*, large changes in expression were not observed for any of the other 44 genes we examined. This was somewhat surprising, particularly because comparative analysis of leaf and root tissue identified 27 differentially expressed genes. Analysis of an expanded set of genes may be required to identify genes whose expression changes upon *HAT4* overexpression; alternatively, a comparison of mRNA populations from specific tissues of wild-type and *HAT4*-transgenic plants may allow identification of downstream genes.

At the current density of robotic printing, it is feasible to scale up the fabrication process to produce arrays containing 20,000 cDNA targets. At this density, a single array would be sufficient to provide gene-specific targets encompassing nearly the entire repertoire of expressed genes in the *Arabidopsis* genome (*2*). The availability of 20,274 ESTs from *Arabidopsis* (*1*, *9*) would provide a rich source of templates for such studies.

The estimated 100,000 genes in the human genome (*10*) exceeds the number of *Arabidopsis* genes by a factor of 5 (*2*). This modest increase in complexity suggests that similar cDNA microarrays, prepared from the rapidly growing repertoire of human ESTs (*1*), could be used to determine the expression patterns of tens of thousands of human genes in diverse cell types. Coupling an amplification strategy to the reverse transcription reaction (*11*) could make it feasible to monitor expression even in minute tissue samples. A wide variety of acute and chronic physiological and pathological conditions might lead to characteristic changes in the patterns of gene expression in peripheral blood cells or other easily sampled tissues. In concert with cDNA microarrays for monitoring complex expression patterns, these tissues might therefore serve as sensitive in vivo sensors for clinical diagnosis. Microarrays of cDNAs could thus provide a useful link between human gene sequences and clinical medicine.

Table 1. Sequences contained on the cDNA microarray. Shown is the position, the known or putative function, and the accession number of each cDNA in the microarray (Fig. 1). All but three of the ESTs used in this study matched a sequence in the database. NADH, reduced form of nicotinamide adenine dinucleotide; ATPase, adenosine triphosphatase; GTP, guanosine triphosphate.

Position	cDNA	Function	Accession number
a1, 2	AChR	Human AChR	*
a3, 4	EST3	Actin	H36236
a5, 6	EST6	NADH dehydrogenase	Z27010
a7, 8	*AAC1*	Actin 1	M20016
a9, 10	EST12	Unknown	U36594†
a11, 12	EST13	Actin	T45783
b1, 2	*CAB1*	Chlorophyll a/b binding	M85150
b3, 4	EST17	Phosphoglycerate kinase	T44490
b5, 6	*GA4*	Gibberellic acid biosynthesis	L37126
b7, 8	EST19	Unknown	U36595†
b9, 10	*GBF-1*	G-box binding factor 1	X63894
b11, 12	EST23	Elongation factor	X52256
c1, 2	EST29	Aldolase	T04477
c3, 4	*GBF-2*	G-box binding factor 2	X63895
c5, 6	EST34	Chloroplast protease	R87034
c7, 8	EST35	Unknown	T14152
c9, 10	EST41	Catalase	T22720
c11, 12	rGR	Rat glucocorticoid receptor	M14053
d1, 2	EST42	Unknown	U36596†
d3, 4	EST45	ATPase	J04185
d5, 6	*HAT1*	Homeobox-leucine zipper 1	U09332
d7, 8	EST46	Light harvesting complex	T04063
d9, 10	EST49	Unknown	T76267
d11, 12	*HAT2*	Homeobox-leucine zipper 2	U09335
e1, 2	*HAT4*	Homeobox-leucine zipper 4	M90394
e3, 4	EST50	Phosphoribulokinase	T04344
e5, 6	*HAT5*	Homeobox-leucine zipper 5	M90416
e7, 8	EST51	Unknown	Z33675
e9, 10	*HAT22*	Homeobox-leucine zipper 22	U09336
e11, 12	EST52	Oxygen evolving	T21749
f1, 2	EST59	Unknown	Z34607
f3, 4	*KNAT1*	*Knotted*-like homeobox 1	U14174
f5, 6	EST60	RuBisCO small subunit	X14564
f7, 8	EST69	Translation elongation factor	T42799
f9, 10	*PPH1*	Protein phosphatase 1	U34803
f11, 12	EST70	Unknown	T44621
g1, 2	EST75	Chloroplast protease	T43698
g3, 4	EST78	Unknown	R65481
g5, 6	*ROC1*	Cyclophilin	L14844
g7, 8	EST82	GTP binding	X59152
g9, 10	EST83	Unknown	Z33795
g11, 12	EST84	Unknown	T45278
h1, 2	EST91	Unknown	T13832
h3, 4	EST96	Unknown	R64816
h5, 6	*SAR1*	Synaptobrevin	M90418
h7, 8	EST100	Light harvesting complex	Z18205
h9, 10	EST103	Light harvesting complex	X03909
h11, 12	*TRP4*	Yeast tryptophan biosynthesis	X04273

*Proprietary sequence of Stratagene (La Jolla, California). †No match in the database; novel EST.

Table 2. Gene expression monitoring by microarray and RNA blot analyses; tg, *HAT4*-transgenic. See Table 1 for additional gene information. Expression levels (w/w) were calibrated with the use of known amounts of human AChR mRNA. Values for the microarray were determined from microarray scans (Fig. 1); values for the RNA blot were determined from RNA blots (Fig. 2).

Gene	Expression level (w/w)	
	Microarray	RNA blot
CAB1	1:48	1:83
CAB1 (tg)	1:120	1:150
HAT4	1:8300	1:6300
HAT4 (tg)	1:150	1:210
ROC1	1:1200	1:1800
ROC1 (tg)	1:260	1:1300

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1. The current EST database (dbEST release 091495) from the National Center for Biotechnology Information (Bethesda, MD) contains a total of 322,225 entries, including 255,645 from the human genome and 21,044 from *Arabidopsis*. Access is available via the World Wide Web (http://www.ncbi.nlm.nih.gov).
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3. D. Shalon, thesis, Stanford University (1995); _____ and P. O. Brown, in preparation. Microarrays were fabricated on poly-L-lysine–coated microscope slides (Sigma) with a custom-built arraying machine fitted with one printing tip. The tip loaded 1 μl of PCR product (0.5 mg/ml) from 96-well microtiter plates and deposited ~0.005 μl per slide on 40 slides at a spacing of 500 μm. The printed slides were rehydrated for 2 hours in a humid chamber, snap-dried at 100°C for 1 min, rinsed in 0.1% SDS, and treated with 0.05% succinic anhydride prepared in buffer consisting of 50% 1-methyl-2-pyrrolidinone and 50% boric acid. The cDNA on the slides was denatured in distilled water for 2 min at 90°C immediately before use. Microarrays were scanned with a laser fluorescent scanner that contained a computer-controlled XY stage and a microscope objective. A mixed gas, multiline laser allowed sequential excitation of the two fluorophores. Emitted light was split according to wavelength and detected with two photomultiplier tubes. Signals were read into a PC with the use of a 12-bit analog-to-digital board. Additional details of microarray fabrication and use may be obtained by means of e-mail (pbrown@cmgm. stanford.edu).
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5. Polyadenylated [poly(A)$^+$] mRNA was prepared from total RNA with the use of Oligotex-dT resin (Qiagen). Reverse transcription (RT) reactions were carried out with a StrataScript RT-PCR kit (Stratagene) modified as follows: 50-μl reactions contained 0.1 μg/μl of *Arabidopsis* mRNA, 0.1 ng/μl of human AChR mRNA, 0.05 μg/μl of oligo(dT) [21-mer], 1× first strand buffer, 0.03 U/μl of ribonuclease block, 500 μM deoxyadenosine triphosphate (dATP), 500 μM deoxyguanosine triphosphate, 500 μM dTTP, 40 μM deoxycytosine triphosphate (dCTP), 40 μM fluorescein-12-dCTP (or lissamine-5-dCTP), and 0.03 U/μl of StrataScript reverse transcriptase. Reactions were incubated for 60 min at 37°C, precipitated with ethanol, and resuspended in 10 μl of TE (10 mM tris-HCl and 1 mM EDTA, pH 8.0). Samples were then heated for 3 min at 94°C and chilled on ice. The RNA was degraded by adding 0.25 μl of 10 N NaOH followed by a 10-min incubation at 37°C. The samples were neutralized by addition of 2.5 μl of 1 M tris-Cl (pH 8.0) and 0.25 μl of 10 N HCl and precipitated with ethanol. Pellets were washed with 70% ethanol, dried to completion in a speedvac, resuspended in 10 μl of H_2O, and reduced to 3.0 μl in a speedvac. Fluorescent nucleotide analogs were obtained from New England Nuclear (DuPont).
6. Hybridization reactions contained 1.0 μl of fluorescent cDNA synthesis product (5) and 1.0 μl of hybridization buffer [10× saline sodium citrate (SSC) and 0.2% SDS]. The 2.0-μl probe mixtures were aliquoted onto the microarray surface and covered with cover slips (12 mm round). Arrays were transferred to a hybridization chamber (3) and incubated for 18 hours at 65°C. Arrays were washed for 5 min at room temperature (25°C) in low-stringency wash buffer (1× SSC and 0.1% SDS), then for 10 min at room temperature in high-stringency wash buffer (0.1× SSC and 0.1% SDS). Arrays were scanned in 0.1× SSC with the use of a fluorescence laser-scanning device (3).
7. Samples of poly(A)$^+$ mRNA (*4*, *5*) were spotted onto nylon membranes (Nytran) and crosslinked with ultraviolet light with the use of a Stratalinker 1800 (Stratagene). Probes were prepared by random priming with the use of a Prime-It II kit (Stratagene) in the presence of [^{32}P]dATP. Hybridizations were carried out according to the instructions of the manufacturer. Quantitation was performed on a PhosphorImager (Molecular Dynamics).
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12. The laser fluorescent scanner was designed and fabricated in collaboration with S. Smith of Stanford University. Scanner and analysis software was developed by R. X. Xia. The succinic anhydride reaction was suggested by J. Mulligan and J. Van Ness of Darwin Molecular Corporation. Thanks to S. Theologis, C. Somerville, K. Yamamoto, and members of the laboratories of R.W.D. and P.O.B. for critical comments. Supported by the Howard Hughes Medical Institute and by grants from NIH [R21HG00450) (P.O.B.) and R37AG00198 (R.W.D.)] and from NSF (MCB9106011) (R.W.D.) and by an NSF graduate fellowship (D.S.). P.O.B. is an assistant investigator of the Howard Hughes Medical Institute.

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Gene Therapy in Peripheral Blood Lymphocytes and Bone Marrow for ADA$^-$ Immunodeficient Patients

Claudio Bordignon,* Luigi D. Notarangelo, Nadia Nobili, Giuliana Ferrari, Giulia Casorati, Paola Panina, Evelina Mazzolari, Daniela Maggioni, Claudia Rossi, Paolo Servida, Alberto G. Ugazio, Fulvio Mavilio

Adenosine deaminase (ADA) deficiency results in severe combined immunodeficiency, the first genetic disorder treated by gene therapy. Two different retroviral vectors were used to transfer ex vivo the human ADA minigene into bone marrow cells and peripheral blood lymphocytes from two patients undergoing exogenous enzyme replacement therapy. After 2 years of treatment, long-term survival of T and B lymphocytes, marrow cells, and granulocytes expressing the transferred ADA gene was demonstrated and resulted in normalization of the immune repertoire and restoration of cellular and humoral immunity. After discontinuation of treatment, T lymphocytes, derived from transduced peripheral blood lymphocytes, were progressively replaced by marrow-derived T cells in both patients. These results indicate successful gene transfer into long-lasting progenitor cells, producing a functional multilineage progeny.

Severe combined immunodeficiency associated with inherited deficiency of ADA (*1*) is usually fatal unless affected children are kept in protective isolation or the immune system is reconstituted by bone marrow transplantation from a human leukocyte antigen (HLA)–identical sibling donor (*2*). This is the therapy of choice, although it is available only for a minority of patients. In recent years, other forms of therapy have been developed, including transplants from haploidentical donors (*3*, *4*), exogenous enzyme replacement (*5*), and somatic-cell gene therapy (*6*–*9*).

We previously reported a preclinical model in which ADA gene transfer and expression successfully restored immune functions in human ADA-deficient (ADA$^-$) peripheral blood lymphocytes (PBLs) in immunodeficient mice in vivo (*10*, *11*). On the basis of these preclinical results, the clinical application of gene therapy for the treatment of ADA$^-$ SCID (severe combined immunodeficiency disease) patients who previously failed exogenous enzyme replacement therapy was approved by our Institutional Ethical Committees and by the Italian National Committee for Bioethics (*12*). In addition to evaluating the safety and efficacy of the gene therapy procedure, the aim of the study was to define the relative role of PBLs and hematopoietic stem cells in the long-term reconstitution of immune functions after retroviral vector–mediated ADA gene transfer. For this purpose, two structurally identical vectors expressing the human ADA complementary DNA (cDNA), distinguishable by the presence of alternative restriction sites in a nonfunctional region of the viral long-terminal repeat (LTR), were used to transduce PBLs and bone marrow (BM) cells independently. This procedure allowed identification of the origin of

C. Bordignon, N. Nobili, G. Ferrari, D. Maggioni, C. Rossi, P. Servida, F. Mavilio, Telethon Gene Therapy Program for Genetic Diseases, DIBIT, Istituto Scientifico H. S. Raffaele, Milan, Italy.
L. D. Notarangelo, E. Mazzolari, A. G. Ugazio, Department of Pediatrics, University of Brescia Medical School, Brescia, Italy.
G. Casorati, Unità di Immunochimica, DIBIT, Istituto Scientifico H. S. Raffaele, Milan, Italy.
P. Panina, Roche Milano Ricerche, Milan, Italy.
*To whom correspondence should be addressed.

cells, and their progeny, after gene transfer.

This combined therapy and marking strategy allowed us to investigate directly in humans some of the basic questions related to the potential of retroviral vectors for gene therapy in cells of the hemato-lymphopoietic lineages. Although gene transfer into human hematopoietic progenitors (*13, 14*), peripheral blood stem cells (*15*), and PBLs (*16–18*) has been extensively demonstrated in vitro, the potential for long-term survival in vivo after the manipulations required for retroviral vector gene transfer remains to be proven. In addition, this study allowed us to study the feasibility of gene transfer into hematopoietic stem and progenitor cells, and the potential for long-term persistence of differentiated cells in a context different from high-dose chemotherapy and BM transplantation (*19–21*). In this system, however, the positive selection may represent an absolute requirement for favoring the appearance of vector-transduced cells.

In ADA$^-$ patients, failure of the immune system to develop is due to the sensitivity of lymphocytes or their precursors to the toxic effects of accumulated ADA substrates (*22*). Because it is possible to reduce the levels of toxic metabolites in ADA$^-$ cells by providing exogenous ADA (*23*), a nonselective form of ADA replacement (that is, transfusion of irradiated red cells from normal individuals) has been used to treat ADA$^-$ patients (*24*). An improved form of treatment was developed by covalent attachment of polyethylene glycol (PEG) to the purified bovine enzyme (*23, 25*). PEGilation appears to block access of degradative enzymes, antibodies, and antigen-presenting cells to the protein surface, thereby inhibiting clearance from the circulation (*26–28*) and prolonging ADA plasma half-life from a few minutes to 24 hours (*23*). The main biochemical consequences of ADA deficiency are almost completely reversed by PEG-ADA treatment (*23*), resulting in an increase in circulating T lymphocytes and improvement of cellular immune functions (*23, 29*).

In our study, treatment in two patients [G.B., patient 1; A.R., patient 2 (*30*); both about 2 years of age] was initiated with weekly intramuscular injections of increasing doses of PEG-ADA (20 and 30 U per kilogram of body weight) until plasma ADA activity could be maintained at least in the normal range of total blood activity. The range of ADA activity was stable between 20 and 40 μmol hour^{-1} ml^{-1}. Before initiation of treatment, both patients had nearly undetectable intracellular ADA activity, and lymphopenia was observed in both patients. Approximately 50% of blood mononuclear cells reacted with monoclonal antibodies to T cell surface antigens, and their proliferative response to mitogens ranged from virtually undetectable to 10% of normal controls. Both patients showed some response in mixed lymphocyte culture, although they produced no specific antibody and showed no antigen-restricted T cell response. Residual immune functions were probably due to previous irradiated red cell transfusions. During the first year of PEG-ADA treatment, lymphocyte counts and proliferative responses to phytohemagglutinin (PHA) normalized.

Immunological reconstitution resulted in increased isohemoagglutinin titer and in cellular and antibody responses to vaccination with tetanus toxoid (TT) (*31*). Associated with reconstitution of immune functions was the complete reversion of all clinical signs of immunodeficiency. However, as reported elsewhere (*29*), the initial reconstitution in this case was limited by the failure to maintain PBL counts and, more markedly, antigen-specific and nonspecific proliferative responses. At that point, the two patients met the conditions that define PEG-ADA treatment failure, as reported in our approved clinical protocol (*12*). Failure of treatment was defined by an extensive number of laboratory parameters and immunological assays (*12*). In both patients, failure of treatment was observed in the absence of any acute illness or open infection episodes and was confirmed in three separate determinations. Waiting for potential recurrence of clinical symptoms such as infectious episodes, or failure of thriving, was considered to be inappropriate.

Early development of T cells obtained during PEG-ADA treatment was crucial to the implementation of the gene therapy protocol. Administration of PEG-ADA continued throughout the study period, although at decreasing amounts. Therefore, the relative role of gene-corrected cells and PEG-ADA treatment remains to be completely defined, an issue that will be addressed during the continuation of this study.

The aim of our study was to evaluate the safety and efficacy of the retroviral vector–mediated gene transfer procedure and to define the relative role of PBLs and BM stem and progenitor cells as effectors of long-term reconstitution of immune functions after gene transfer. For this purpose, we constructed two different retroviral vectors, DCA*l* and DCA*m*, expressing the human ADA cDNA under the control of its own promoter, which were used to infect PBLs and BM cells, respectively (Fig. 1). Both vectors are based on the double-copy (DC) design (*32*) and are structurally identical except for the presence of alternative restriction sites (Mlu I in DCA*l* and Bss HII in DCA*m*) in a nonfunctional region of the viral LTR (*33*). This feature allowed unequivocal tracing of the origin (BM or PBL) of the transduced cell progeny in the circulation by a simple polymerase chain reaction (PCR) analysis on genomic DNA (*34*). Both vectors were packaged in the amphotropic GP+*env* Am12 cell line (*33*). PBLs and T cell–depleted BM cells were transduced ex vivo either by multiple exposure to cell-free viral supernatant or by coculture with irradiated packaging cells (*35, 36*). Gene transfer efficiency increased from 1 to 2.5% up to 40% in total PBLs, with the introduction in the procedure of a new packaging line and of cocultivation. Gene transfer efficiency into CFU-GM and BFU-E hematopoietic progenitors averaged 30 to 40%, as described (*37*). These frequencies were estimated by cloning in lim-

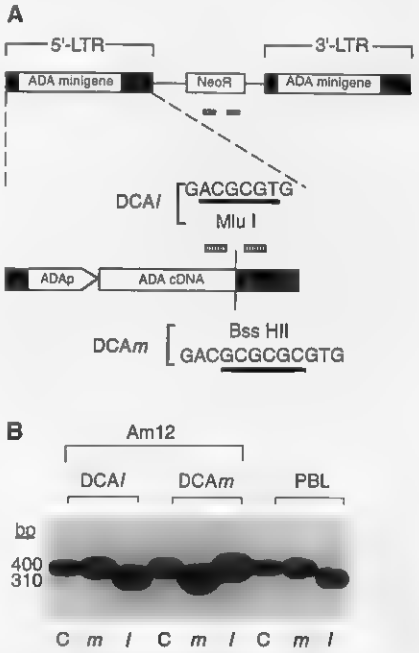

Fig. 1. (**A**) Structure of the DCA*l* (lymphocytes) and DCA*m* (marrow) proviruses. A human ADA minigene (promoter + full-length cDNA) was inserted into the LTR U3 region of a Moloney murine leukemia virus–derived retroviral vector (DCA) (*32, 33*). For construction of two vectors that could be distinguished from each other after integration into the target cell genome, the unique Mlu I restriction site present in a functionally irrelevant region of the LTR in DCA*l* was converted into a Bss HII site in DCA*m* (enlarged map). The hatched boxes indicate the location of the PCR primers used to detect vector DNA in target cells and for vector identification. (**B**) PCR identification of the vector integrated into the lymphocytes of patient 1 3 months after initial administration of DCA*l*-transduced PBLs and DCA*m*-transduced BM cells, showing the PBL origin of the transduced circulating lymphocytes. C, control (uncut PCR product); *m*, marrow-specific Bss HII cut present in DCA*m*-transduced cells; *l*, lymphocyte-specific Mlu I cut present in DCA*l*-transduced cells. PCR amplification of DNA obtained from the DCA*l* and DCA*m* packaging cell lines (Am12) is shown as a control.

iting dilution (38) and semi-solid colony-forming assays (39), respectively, in the presence or absence of G418 and are the result of the steady improvement in both cell-free infection and cocultivation that we have produced in recent years (*10, 11, 18, 37*). In particular, our goal has been to increase gene transfer frequency while maintaining phenotype, immune repertoire, and in vivo potential for proliferation, differentiation, and survival. For this purpose, short cultivation time under conditions of low interleukin-2 (IL-2) concentration were developed for the activation and infection of PBLs (35), while BM cells were maintained in a long-term culture system over adherent layers without addition of exogenous growth factors, and were infected during the first 3 days of culture (36). This system produces minimal loss of differentiation capacity and potential for in vivo hematopoietic reconstitution (*40*). No G418 selection was applied to infected PBLs or BM cells before reinfusion. Transduction efficiency and production of the vector-derived ADA in infected cells was determined by PCR and thin-layer chromatography (TLC), respectively (*41*).

In vivo administration of genetically modified cells began in March 1992 for patient 1 and July 1993 for patient 2. Patient 1 received 7.24×10^8 DCA*l*-transduced lymphocytes and 0.35×10^8 DCA*m*-transduced progenitor cells in nine injections administered intravenously (i.v.) over a period of 24 months. Patient 2 received a slightly smaller number of cells in five injections over 10 months.

We began monitoring the persistence of vector-transduced cells at monthly (or bimonthly) intervals from the first infusion. Analyses were performed both on bulk populations of cells of different origin, for the indication of origin of transduced cells, and on clonal assays for quantitation of transduced BM and PBLs. Six months after the beginning of treatment, long-term survival of transduced cells was demonstrated in both patients by the presence of vector-derived sequences in the DNA extracted from peripheral blood mononuclear cells, total BM cells, mature granulocytes (Fig. 2A), individual T lymphocyte clones (Fig. 3), and BM progenitors (BFU-E, CFU-GM, and CFU-GEMM) in clonal culture (*41*). ADA production at levels substantially greater than observed in untransduced ADA controls was observed in PBLs, BM, and granulocytes (Fig. 2C). The proportion of genetically modified cells in BM and circulating blood was monitored throughout the study by BM and T cell clonal assay in the presence of G418, and indirectly from the amount of ADA activity in total cell populations from BM and peripheral blood. In both patients, this proportion ranged between 5 and 30% of clonable BM progenitors and between 0.8 and 8.5% in PBLs. Total ADA activity ranged between 5 and 18% of normal values in both patients' nucleated cells in the blood. Sixteen months after discontinuation of treatment, ADA activity in total circulating nucleated cells was 126 nmol hour^{-1} per milligram of protein in patient 1 and 77 nmol hour^{-1} per milligram of protein in patient 2 (internal normal control, 1157 nmol hour^{-1} per milligram of protein; for method see legend to Fig. 2). At the same time, in patient 1, the frequency of transduced G418-resistant T cell was 4.76% and that of clonable BM progenitors was 25%; in patient 2 these frequencies were 2.01 and 17%, respectively. During this period in patient 1, and more recently in patient 2, ADA activity became reproducibly detectable also in circulating erythrocytes (Fig. 2C). Vector-derived ADA activity in individual T cell clones was comparable to, or higher than, that of normal controls (legend to Fig. 2C), as observed in T cells that survived in vivo selection in the human PBL-SCID mouse preclinical model (*10, 11*). ADA activity in Neo-resistant BM colonies also averaged normal levels (*41*).

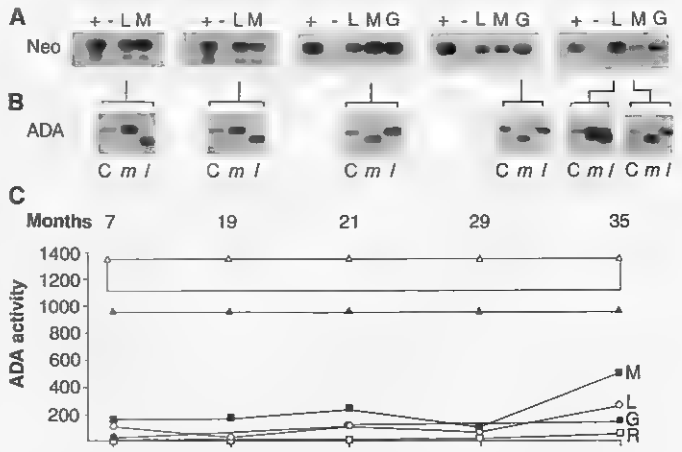

Fig. 2. Persistence of transduced hematopoietic cells in vivo and analysis of their origin. During 3 years after initiation of the gene therapy trial, persistence of transduced PBLs and BM cells, and expression of vector-derived ADA activity were documented at regular intervals. (**A**) Detection of transduced cells by PCR analysis for the NeoR gene was consistent throughout the follow-up of patient 1 in PBLs (L), BM cells (M), and circulating granulocytes (G). + and −, PCR positive and negative controls, respectively. (**B**) Analysis of the identity of the integrated vector showed that vector-positive lymphocytes were initially all derived from long-lived transduced PBLs, as demonstrated by the presence of the DCA*l*-specific PCR pattern (7 and 19 months), whereas BM and granulocytes showed the DCA*m*-specific pattern (21, 29, and 35 months). Three years after initiation and 1 year after discontinuation of treatment, DCA*m*-specific signals started to appear in the DNA extracted from PBLs, indicating progressive conversion of the circulating, genetically modified lymphocyte pool from a predominantly PBL-derived to a BM progenitor–derived population (35 months). This observation was further confirmed by the analysis of Neo-resistant, peripheral blood T cell clones (Fig. 3). (**C**) In parallel, vector-derived ADA activity was monitored by TLC in total PBLs (L) and BM cells (M) of patient 1. G, granulocytes; R, red blood cells. Two positive controls are provided: ADA activity (mean ± SE) from a pool of normal individuals (△), and ADA activity from a polyclonal PBL line from the same patient transduced in vitro and selected in G418 (▲). Lysates were prepared from 1×10^6 cells in 10 μl of CGLB buffer by three cycles of freeze and thaw. ADA enzyme activity was analyzed by the ^{14}C-adenosine to ^{14}C-inosine conversion assay followed by TLC (*37*). Cell lysates from individual clones (~2×10^5 cells) were normalized for protein content by the BIO-RAD protein assay (Bio-Rad Laboratories GmbH, Munich, Germany). Positive and negative controls were, respectively, lysates from normal PBLs and uninfected, IL-2–stimulated ADA PBLs, because IL-2 stimulation is reported to increase the efficiency of ADA expression in ADA cells (*37*). TLC plates were exposed for 3 days in a PhosphorImager (Molecular Dynamics, Sunnyvale, California). Ratio of adenosine conversion is expressed as nmol hour^{-1} mg^{-1}.

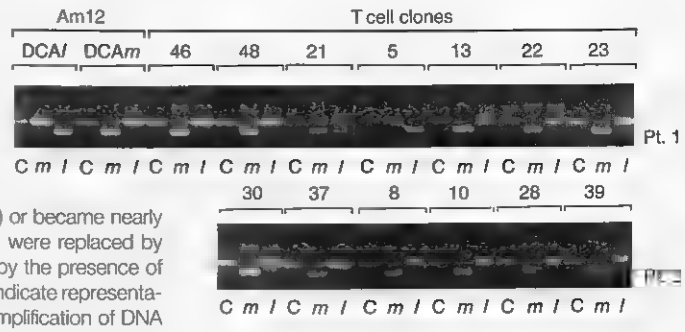

Fig. 3. Origin of T cell clones obtained from the peripheral blood of patient 1 (top) and patient 2 (bottom) 1 year after discontinuation of treatment. Clonable T cells containing the DCA*l* vector diminished markedly (patient 1) or became nearly undetectable (patient 2), and were replaced by BM-derived T cells, marked by the presence of the DCA*m* vector. Numbers indicate representative individual clones. PCR amplification of DNA obtained from the DCA*l* and DCA*m* packaging cell lines (Am12) is shown as a control.

Spontaneous revertants (ADA-positive, vector-negative) were not observed in either peripheral blood or BM.

Initially, the analysis of the retroviral vector amplified from the DNA of circulating lymphocytes indicated that genetically modified cells were derived from a pool of long-lived PBLs originally transduced with the DCA*l* vector (Fig. 1B). This finding was consistent throughout the period of administration of transduced PBLs and BM cells (Fig. 2B, at 7 and 19 months, for example), whereas total BM cells (Fig. 2B, at 21 and 35 months, for example) and circulating granulocytes (Fig. 2B, at 29 months, for example) always showed the DCA*m*-specific restriction pattern or marrow-derived cells. However, about 1 year after discontinuation of gene therapy, both PBL- and BM-derived lymphocytes were detectable in the circulation (Fig. 2B, at 35 months). At that time, Neo-resistant, clonable T cells containing the PBL-specific DCA*l* vector sharply decreased (Fig. 3, patient 1) or became undetectable (Fig. 3, patient 2) and were progressively replaced in the circulation by T cells containing the BM-specific, DCA*m* vector. To confirm this important finding, we evaluated two additional time points, subsequent to the data in Figs. 2 and 3, on bulk populations and on T lymphocyte clones. Thirty-eight clones from patient 1 were analyzed for their origin; 26 were derived from marrow, 6 could not be unequivocally determined, and 9 contained the DCA*l* vector. Similarly, of 49 clones obtained from patient 2, 6 could not be clearly determined, 6 contained the DCA*l* vector, and all others were derived from marrow.

These results show that short-term immune reconstitution was sustained in the two patients by a population of peripheral blood–derived, ADA-producing lymphocytes with a life-span in the circulation ranging between 6 and 12 months. We have previously shown that this population contains both mature T cells and immature, or naïve, precursors (*11*, *18*). Conversely, long-term reconstitution resulted almost exclusively from transduced, BM-derived hematopoietic stem and progenitor cells capable of generating multilineage progenies of ADA-producing cells, that is, lymphocytes, granulocytes, and (more recently) erythrocytes.

A fundamental hypothesis underlying this study was the possibility that genetically corrected cells would benefit from a selective advantage over noncorrected cells. Our experimental design has made it possible to obtain data in support of this hypothesis. The first line of evidence comes from the progressive appearance of marrow-derived PBLs, generated over time from a relatively small number of genetically modified precursors contained in the transduced marrow cell population (Figs. 2 and 3). Additional evidence comes from the analysis of the integrated retroviral vectors. In a recent comparative analysis of different vector constructs designed for gene transfer of reporter genes in human PBLs, we demonstrated that the DC construct carries an inherent instability that results in loss of the gene inserted in the viral LTR (*18*). Such instability could affect 50% of integrated proviruses, depending on the size and nature of the inserted gene. In the present study, the analysis of over 200 T cell clones obtained at different times during the follow-up of the two patients showed no rearrangement that might have eliminated the ADA gene, and consequently its expression. Conversely, loss of the ADA gene could be detected only in marrow-derived colonies and T cell clones that had been transduced and cultured in vitro, in the absence of any positive selection (*41*). These observations indicate that, in ADA$^-$ SCID patients, ADA-producing cells have a selective advantage over noncorrected ADA$^-$ cells, as previously suggested in the

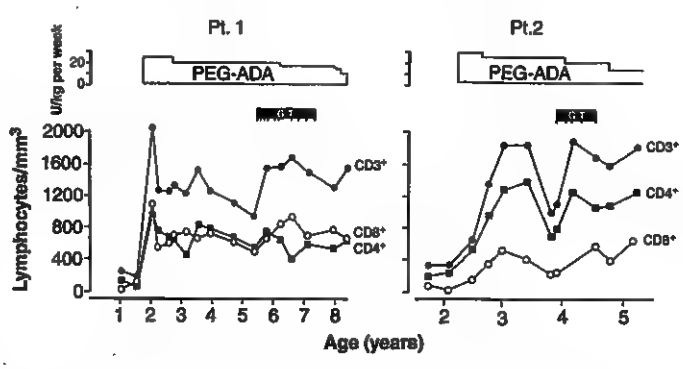

Fig. 4. Immune reconstitution during the PEG-ADA and gene therapy trial for patient 1 (left) and patient 2 (right). CD3$^+$, CD4$^+$, and CD8$^+$ lymphocyte counts are plotted against age for the duration of the trial. Doses of PEG-ADA administered to the patients are shown in the upper part of the graphs. The black boxes (GT) indicate the period of administration of genetically modified PBLs and BM cells (ticks indicate individual injections).

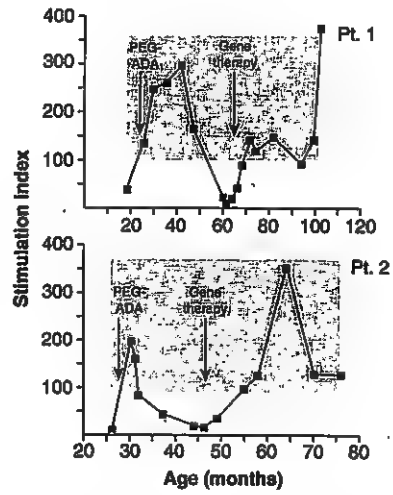

Fig. 5. Immune reconstitution during the PEG-ADA and gene therapy trial for patient 1 (top) and patient 2 (bottom). T cell proliferative response to mitogenic stimulus is presented as stimulation index (cpm of stimulated samples divided by cpm of unstimulated cells) and is plotted against age of the patients. Shaded areas indicate the range of the stimulation index of normal internal controls. Response to TT followed a comparable kinetics. Arrows indicate initiation of enzyme replacement therapy (PEG-ADA) and administration of genetically modified cells (gene therapy).

Table 1. Quantitation of isoagglutinin titer, and antigen-specific antibody production and proliferative response after vaccination with tetanus toxoid (TT). IgG, immunoglobulin G; ND, not done.

Time of test	Anti-B isoagglutinin titer		Serum titer of anti-TT IgG*		Proliferative response (10^3 cpm)†	
	Patient 1	Patient 2	Patient 1	Patient 2	Patient 1	Patient 2
Before immunization	ND	1/2	2.4	0.2	0.5	1.6
PEG-ADA response	1/8	1/16	1600	130	26.4	15.7
PEG-ADA failure	1.8	1.5	ND	34	1.8	1.5
After gene therapy I	1/16	1/32	ND	88	50.3	67.6
After gene therapy II	1/32	1/32	ND	ND	82.2	96.5

*Anti-TT IgG production was determined in a standard enzyme-linked immunosorbent assay and is reported as international units to a reference standard (Biagini Reference Standard, Biagini, Florence, Italy). †TT-specific T cell lines were tested for their capacity to proliferate in response to TT in the presence of autologous antigen-presenting cells (*45*). Proliferation in response to antigen-presenting cells alone was always <1000 cpm. Both patients received the full immunization schedule with TT (three doses) while on PEG-ADA. Patient 2 showed signs of immune deterioration during the immunization schedule. Isoagglutinin titer, TT-specific IgG titer, and TT-specific T-cell proliferation were measured at the following times: before immunization and before use of PEG-ADA (2 years and 5 months of age for patient 1 and 2 years and 10 months for patient 2), at the time of peak response to PEG-ADA (2 years since the beginning of PEG-ADA treatment for patient 1 and 1 year for patient 2), at PEG-ADA failure, and twice after gene therapy (6 and 8 years of age for patient 1 and 4.5 years and 5 years for patient 2).

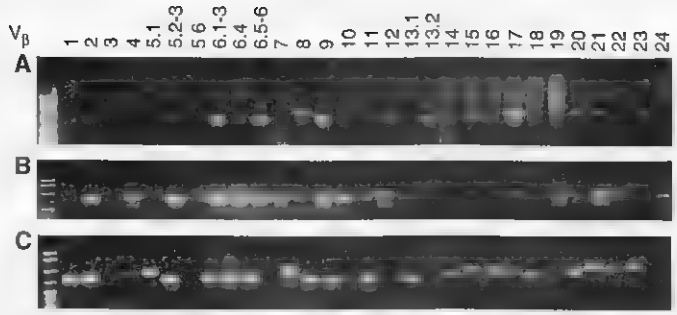

Fig. 6. Development of a normal T cell receptor repertoire in patient 1 after gene therapy treatment. Different T cell receptor V_β-chain usage at the time of failure of the PEG-ADA treatment (**A**), 1 year after the beginning of gene therapy (**B**), and 1 year after discontinuation of transduced cell administration (**C**), as analyzed by RT-PCR amplification with V_β chain-specific primers (*48*).

human PBL–SCID mouse preclinical model (*10, 11*).

Immune reconstitution induced by PEG-ADA treatment lasted for over 3 years in patient 1 and for a shorter period in patient 2, despite administration of a 50% higher PEG-ADA dose in the latter (Figs. 4 and 5). In association with a progressive decline in PBL counts, the immune response decreased markedly over a short period of time (Figs. 4 and 5 and Table 1). Administration of genetically modified cells rapidly restored immune functions in both patients, resulting in normalization of total lymphocyte counts (Fig. 4) and cellular and humoral responses, including sustained isohemoagglutinin titer, antigen-specific antibody production, and mitogen- and antigen-specific proliferation (Fig. 5 and Table 1). The T cell receptor repertoire, analyzed by the V_β chain usage, normalized progressively (Fig. 6). In patient 2, the overall response to gene therapy was similar to that of patient 1, despite administration of a smaller number of genetically modified PBLs and BM cells. There have been no serious infections in the two patients throughout the PEG-ADA treatment and after the beginning of gene therapy. The patients received no other treatment, except for high-dose immunoglobulins administered intravenously and prophylactic antibiotic treatment, both of which were discontinued after indications of full immunologic reconstitution. Before the beginning of the PEG-ADA treatment, the patients showed severe growth failure, ranging below the fifth percentile for height and weight. Enzyme replacement and gene therapy had a marked clinical impact, resulting in normalization of height and weight. Patient 2, who had a very limited initial response to PEG-ADA, resumed normal growth only after gene therapy. Serum chemistry values, blood counts, and urinalysis indicated no toxicity from PEG-ADA or gene therapy treatments. Monitoring of the two patients for the presence of recombinant helper virus was consistently negative.

The results of the long-term follow-up have two main implications: the selection of an optimal treatment for ADA$^-$ SCID patients and, more generally, the potential application of similar gene therapy approaches to the treatment of genetic and acquired diseases. Our study clearly indicates the feasibility of direct BM cell gene therapy; however, in specific circumstances, genetically modified PBLs may provide a prompt supply of immune effector cells until development of BM-derived lymphocytes. If it is proven to be efficacious over time, this procedure could represent a less toxic alternative to unrelated or HLA-mismatched marrow transplants. In the prospective extension of these results to the design of other gene therapy clinical trials, gene transfer into hematopoietic progenitors can be achieved also in the absence of the stress conditions associated with cytoreduction and BM transplantation. However, in steady-state hematopoiesis a considerable time lag may be required before appearance of genetically modified cells in the blood. Under these conditions, the positive selection may represent an absolute requirement for favoring the appearance of vector-transduced cells. Such positive selection may be "naturally" present in other genetic or acquired diseases [for example, acquired immunodeficiency syndrome (AIDS)] or could be built into the vector as a drug-resistance gene.

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31. Both patients were vaccinated with tetanus toxoid (TT; Swiss Serum Institute, Bern, Switzerland). To evaluate the number of precursor lymphocytes specific to TT, the frequency of cells capable of proliferating in the presence of irradiated autologous PBLs that had been pulsed overnight with TT was evaluated in a limiting dilution assay. At two different times after vaccination, the frequency of TT-specific lymphocyte precursors was 1:2500 and 1:5000 in patient 1. This range is comparable to that of normal individuals at the same time after immunization. Patient 2 showed signs of immune deterioration during the immunization schedule (Table 1).
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33. The double-copy DCA retroviral vector, carrying a human ADA minigene, has been described previously (*32*) and is indicated as DCA*I* in this report. The DCA*m* vector was obtained by digestion, Klenow-filling, and religation of the DCA*I* plasmid at the Mlu I restriction site in the polylinker region of the 3′ LTR, thus generating a new Bss HII site. The Am12/DCA*m*-7 and the Am12/DCA*I* -18 clonal packaging cell lines were generated by the transinfection protocol, as described (*18*).
34. High molecular weight DNA was obtained from cells by standard phenol-chloroform extraction (*42*). A Neo-specific, 362-bp fragment located in the coding region of the NeoR gene or a DCA-specific 400-bp fragment spanning the 3′ end of the ADA cDNA, the polylinker, and part of the LTR U3 region, were amplified from 0.5 μg of genomic DNA by 30 cycles of PCR with 5 U of Taq polymerase (Perkin Elmer, Norwalk, CT) and 25 pmol of the primers Neo-1 (5′-GGAAGCCGGTCTTGTCGATC-3′), Neo-3 (5′-AGAGTCCCGCTCAGAAGAAC-3′), DCA-5 (5′-TCAATGCGGCCAAATCTAG-3′), and DCA-6 (5′-GCTGTTCCATCTGTTCCTGA-3′). DCA-specific PCR products were digested by Bss HII and Mlu I restriction enzymes (Boehringer Mannheim GmbH, Mannheim, Germany). Reaction mixtures (1/10th of the total volume) were separated on a 1.5% agarose gel, and DNA was visualized by ethidium bromide staining. DNA was transferred to a nylon membrane (Hybond-N, Amersham, Buckinghamshire, UK) by DNA capillary blotting (*42*) and hybridized to 10^7 dpm of ^{32}P-labeled, 1.2-kb Hind III–Sma I fragment of pSV2-neo (*43*), or 1.9 Kb Xho I fragment of the ADA cDNA. Filters were washed under high-stringency conditions and exposed to Kodak X-AR5 film for 30 min to overnight at −70°C.
35. Freshly isolated ADA$^-$ PBLs were obtained from ADA SCID patients, Ficoll-fractionated, and grown at 10^6 cells/ml in 24-well tissue culture plates under phytohemagglutinin (PHA) (2 μg/ml; Boehringer Mannheim GmbH) and human recombinant IL-2 (hu-rIL-2, 100 U/ml; Eurocetus B.V., Amsterdam, Netherlands) stimulation in lipopolysaccharide-free RPMI 1640 medium supplemented with 2 mM L-glutamine and 5% human serum. In subsequent experiments similar levels of gene transfer could be obtained at 50 or 100 U of hu-rIL-2 per milliliter, in the absence of any additional stimulus (C. Benati *et al.*, in preparation). After 72 to 96 hours of stimulation, T lymphocytes were cocultivated with irradiated (10,000 roentgen) vector-producing cells for 72 hours in complete Dul-

becco's minimum essential medium (DMEM) in the presence of polybrene (8 μg/ml) as described (*18*). Cells were washed twice in phosphate-buffered saline (PBS), resuspended in fresh medium, and cultured for 3 to 4 days. Transduced cells were tested for the presence of helper virus and cryopreserved until use.
36. BM mononuclear cells were obtained as a Ficoll fraction and grown for 2 to 3 days in complete DMEM at a density of 6×10^5 to 8×10^5 cells/cm^2 (*35*). T cell depletion and progenitor cell enrichment were obtained as described (*3, 35*). Gene transfer was carried out by multiple infection cycles with cell-free, helper virus–tested viral supernatants in the presence of polybrene (8 μg/ml) (*35*). BM cells were maintained in a long-term culture system over adherent layers without addition of exogenous growth factors, and infected during the first 3 days of culture. Transduced cells were tested for the presence of helper virus and cryopreserved until use. At that time, the transduced cells were washed, resuspended in normal saline containing 4% human albumin, and reinfused into the patient.
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38. PHA blasts or antigen-specific T cells were cloned by limiting dilution. The relative frequencies of transduced cells was obtained by comparing the precursor frequency in the absence and presence of G418 (800 μg/ml). G418-resistant T cell clones were isolated and maintained as described (*44, 45*).
39. The relative frequencies of transduced BM progenitor cells were obtained by comparing the frequency of CFU-G, CFU-GM, BFU-E, and CFU-GEMM cells in the absence and presence of increasing doses of G418 (0.7, 1.0, 1.5 mg/ml) as described (*37*). In selected experiments, individual G418-resistant colonies were collected for analysis of vector transduction and expression.
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48. T cell receptor V_β-chain usage was analyzed on transduced T cell lines by reverse transcriptase–PCR. Briefly, total RNA was reverse transcribed with oligo(dT) and oligo(dG) primers and subjected to PCR with V_β- or C_β-specific oligonucleotides (*46*) or to anchored PCR with a C_β-specific oligonucleotide as described (*47*). Amplified products were analyzed by agarose gel electrophoresis.
49. We are indebted to L. Ruggieri and A. Wack for performing some of the ex vivo and in vitro analyses of gene transfer frequency; to the nurses and clinical staff of the Clinica Pediatrica, School of Medicine, University of Brescia, for skilled and dedicated care; to A. Arrighini and A. Crescenzo for clinical assistance in the extended care of the two patients; to A. Plebani for dosing specific antibody production; to M. Hershfield, P. Dellabona, and A. Ballabio for helpful discussions; and to Enzon, Inc., and Ophan Europe for providing PEG-ADA before commercial distribution. Supported by grants from Telethon, the Italian National Research Council, and the Italian Ministry of Health (IV-VII AIDS Projects).

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T Lymphocyte–Directed Gene Therapy for ADA$^-$ SCID: Initial Trial Results After 4 Years

R. Michael Blaese,* Kenneth W. Culver, A. Dusty Miller, Charles S. Carter, Thomas Fleisher, Mario Clerici,† Gene Shearer, Lauren Chang, Yawen Chiang, Paul Tolstoshev, Jay J. Greenblatt, Steven A. Rosenberg, Harvey Klein, Melvin Berger, Craig A. Mullen,‡ W. Jay Ramsey, Linda Muul, Richard A. Morgan, W. French Anderson§

In 1990, a clinical trial was started using retroviral-mediated transfer of the adenosine deaminase (ADA) gene into the T cells of two children with severe combined immunodeficiency (ADA$^-$ SCID). The number of blood T cells normalized as did many cellular and humoral immune responses. Gene treatment ended after 2 years, but integrated vector and ADA gene expression in T cells persisted. Although many components remain to be perfected, it is concluded here that gene therapy can be a safe and effective addition to treatment for some patients with this severe immunodeficiency disease.

The possibility of using gene transfer as a therapy for human disease has great appeal. The decision to enter clinical trials awaited the development of safe and efficient techniques of gene transfer and improved understanding of the basic pathology and biology underlying likely candidate diseases and target cells. The advent of useful retroviral vectors that permitted relatively high efficiency gene transfer and stable integration was a critical advance (*1*, *2*), as was the demonstration that this procedure of gene transfer could be effectively and safely used in humans (*3*).

Severe combined immunodeficiency secondary to a genetic defect in the purine catabolic enzyme adenosine deaminase [ADA$^-$ SCID] is characterized by defective T and B cell function and recurrent infections, often involving opportunistic pathogens. Large amounts of deoxyadenosine, an ADA substrate, are present in these patients; deoxyadenosine is preferentially converted to the toxic compound deoxyadenosine triphosphate in T cells, disabling the immune system (*4*).

Because this disease is curable by allogeneic bone marrow transplantation given without pretransplantation cytoreductive conditioning, it was initially assumed that gene therapy should be directed at the bone marrow stem cell. However, initial attempts to use stem cell gene transfer in primates resulted in only low-level, transient gene expression, insufficient for clinical use. The observation that the only donor cells detected in some patients "cured" by allogeneic bone marrow transplantation was their T cells—the others remaining ADA-deficient (*5*)—raised the possibility that T cell–directed gene therapy also might be a useful treatment.

The introduction of enzyme replacement with ADA-containing erythrocytes (*6*) or with bovine ADA conjugated with polyethylene glycol (PEG-ADA) (*7*) has made this approach feasible. PEG-ADA has provided noncurative, life-saving treatment for ADA$^-$ SCID patients; with this treatment, most patients have experienced weight gain and decreased opportunistic infections. Full immune reconstitution has been less regularly achieved with enzyme therapy. T cell function as measured by in vitro mitogen responses improved in most patients, but fewer patients recovered consistent immune responses to specific antigens [for instance, as measured by normal delayed-type hypersensitivity (DTH) skin test reactivity] (*8–10*). Nearly all PEG-ADA–treated patients showed increased peripheral T cell counts, which provided a source of T cells for gene correction not available without enzyme therapy. Furthermore, enzyme treatment could be continued during the gene therapy trial so that the ethical dilemma of withholding or stopping a life-saving therapy to test an unknown treatment could be avoided.

The adenosine deaminase complementary DNA (cDNA) (*11*) is 1.5 kb and fits within a retroviral vector. With the use of an ADA-containing retroviral vector, ADA-deficient T cell lines were transduced to express normal amounts of ADA; this rendered them normally resistant to intoxication and growth inhibition when challenged with deoxyadenosine (*12*, *13*). Next, studies in mice, rabbits, and nonhuman primates using T cells modified with retroviral vectors showed normal cell survival and function after their reintroduction into recipient animals (*14*). Finally, Bordignon and colleagues (*15*) showed that ADA gene–corrected T cells acquired a survival advantage compared with uncorrected ADA-deficient cells when transplanted into immunodeficient, but ADA-

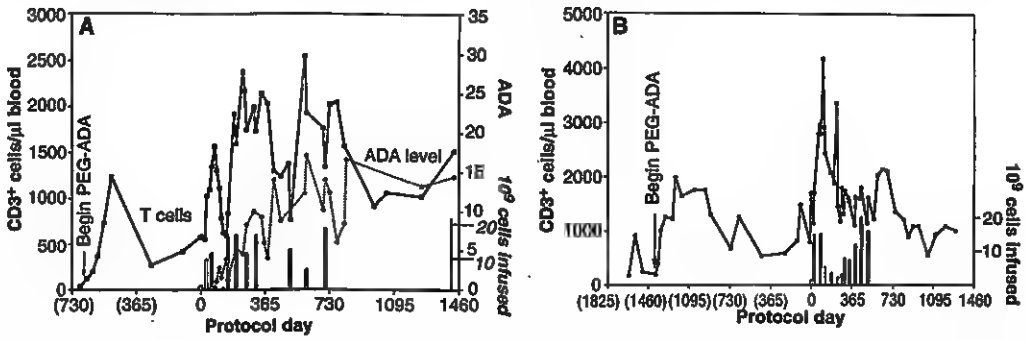

Fig. 1. Peripheral blood T cell counts since the time the diagnosis of ADA deficiency was made, dates of treatments, and the total number of cells infused for each patient. ADA level is measured in nanomoles of adenosine deaminated per minute per 10^8 cells. Vertical bars indicate the dates of cell infusion, and their height represents the total number of nonselected cells infused at each treatment. The T cell numbers represent total CD3-bearing T cells determined by standard flow cytometric analysis. (**A**) Patient 1 began gene therapy on 14 September 1990 (protocol day 0) and received a total of 11 infusions. Cellular ADA enzyme level is indicated by the dashed line. ADA activity was determined as described (*13, 25*). Values shown are the mean of duplicate samples and represent EHNA-sensitive ADA enzyme activity. (**B**) Patient 2 began gene therapy on 31 January 1991 (protocol day 0) and received of a total of 12 infusions.

normal BNX recipient mice.

The clinical protocol used here has been described elsewhere (*16*). Patients with documented ADA$^-$ SCID were eligible if they did not have a human lymphocyte antigen–matched sibling as a potential donor for marrow transplantation and if they had been treated with PEG-ADA for at least 9 months without full immune reconstitution. T cells were obtained from their blood by apheresis, induced to proliferate in culture, transduced with the ADA retroviral vector LASN, culture-expanded, and then reinfused into the patient after 9 to 12 days (*17*). No selection procedure was used to enrich for gene-transduced cells.

The clinical histories and ADA gene mutations of each patient have been reported (*18, 19*). Patient 1 presented with infection at 2 days of age and had recurrent infections and very poor growth until 26 months of age, when the diagnosis of ADA deficiency was established and she was started on PEG-ADA [30 U per kilogram of body weight per week (30 U/kg/week)]. Treatment with PEG-ADA enzyme for approximately 2 years had resulted in significant, but incomplete, benefit. With PEG-ADA she gained weight, had fewer infections, and transiently developed a normal peripheral blood T cell count (Fig. 1A), and her T cells had acquired the ability to respond to mitogens in vitro. However, significant immune deficiency persisted, including recurrence of her T lymphopenia (Fig. 1A), DTH skin test anergy (Table 1), depressed in vitro immune reactivity to specific antigens such as tetanus toxoid, failure to generate normal cytotoxic T cells to viral antigens or allogeneic cells, defective immunoglobulin production and absent or weak antibody responses to several vaccine antigens, and borderline isohemagglutinin titers (Table 1). At 4 years of age, she was enrolled in this trial.

The course of disease in patient 2 (who was 9 years old when enrolled in the trial) was milder than that seen in classic SCID (*19*). She had her first serious infection at age 3, and septic arthritis at age 5; the diagnosis was finally established at age 6 when significant lymphopenia with ADA deficiency was confirmed. This patient had an excellent initial improvement in peripheral T cell numbers after the start of PEG-ADA therapy (30 U/kg/week) at age 5, but lymphopenia recurred in the third and fourth years of enzyme treatment (Fig. 1B). During the year before gene therapy, repeated evaluation of her immune system showed persisting immunodeficiency, but less severe than that in patient 1. Despite 4 years of enzyme treatment, DTH skin test reactivity was absent (Table 1), cytotoxic T cells to viral antigens and allogeneic cells were deficient, and isohemagglutinins were barely detectable. However, illustrating the variability seen in the responses of patient 2 over time, blood lymphocytes that were cryopreserved from the day the clinical trial began and tested later showed normal cytotoxic activity to allogeneic cells.

Within 5 to 6 months of beginning gene therapy, the peripheral blood T cell counts for patient 1 (Fig. 1A) rapidly increased in number and stabilized in the normal range and have remained normal since that time (*20*). ADA enzyme activity, nearly undetectable in her blood lymphocytes initially, progressively increased in concentration during the first 2 years of treatment to reach a level roughly half the concentration found in heterozygous carriers (expressing only one intact ADA allele) and has re-

Table 1. DTH skin test reactivity and isohemagglutinin titers in sera of each patient at various times during the treatment protocol. Skin tests were applied as Multitest (Pasteur Merieux, Lyon, France) and scored according to the manufacturer's instructions 48 to 72 hours after being placed. Seven antigens were placed on the dates indicated, although only five were technically satisfactory on day 1252 for patient 1 and on day 1118 for patient 2. Isohemagglutinin titers were determined by standard blood bank techniques (*34*). Ninety five percent of normal children over the age of 2 years will have a titer of ≥1:16 and 82% will have a titer ≥1:32 (*35*). ND, not done. For the DTH skin tests, positive tests were elicited; T, tetanus toxoid; D, diphtheria toxoid; C, *Candida albicans*; P, Proteus antigen; S, streptococcal antigen; OT, old tuberculin.

Protocol day	Isohemagglutinins	DTH skin tests
	Patient 1	
−9	16	None (0/7)
115	256	ND
251	128	ND
314	32	T, D, C
455	32	T, D, C, S, P
510	64	ND
707	32	ND
1252	ND	D, C, P
	Patient 2	
−122	4	None (0/7)
−9	4	ND
90	256	ND
186	128	ND
291	128	ND
501	128	T, D, C, S, OT
676	64	ND
957	16	ND
1118	ND	T, D, S, P

R. M. Blaese, C. A. Mullen, W. J. Ramsey, National Center for Human Genome Research, National Institutes of Health (NIH), Building 49, Room 2A03, Bethesda, MD 20892, USA, and National Cancer Institute (NCI), NIH, Bethesda, MD 20892, USA.
K. W. Culver, M. Clerici, G. Shearer, J. J. Greenblatt, S. A. Rosenberg, NCI, NIH, Bethesda, MD 20892, USA.
A. D. Miller, Fred Hutchinson Cancer Research Center, Seattle, WA 98104, USA.
C. S. Carter, T. Fleisher, H. Klein, Clinical Center, NIH, Bethesda, MD 20892, USA.
L. Chang and W. F. Anderson, National Heart, Lung, and Blood Institute (NHLBI), NIH, Bethesda, MD 20892, USA.
Y. Chiang and P. Tolstoshev, Genetic Therapy, Gaithersburg, MD 20878, USA.
M. Berger, Department of Pediatrics, Case Western Reserve University School of Medicine, Cleveland, OH 44106, USA.
L. Muul, National Center for Human Genome Research, NIH, Bethesda, MD 20892, USA.
R. A. Morgan, National Center for Human Genome Research, NIH, Bethesda, MD 20892, USA, and NHLBI, NIH, Bethesda, MD 20892, USA.

*To whom correspondence should be addressed at the National Center for Human Genome Research. E-mail: mblaese@nchgr.nih.gov
†Present address: Cattedra Di Immunogia, Universita Milano, Milan 20133, Italy.
‡Present address: Division of Pediatrics, M. D. Anderson Cancer Center, Houston, TX 77030, USA.
§Present address: Norris Cancer Center, University of Southern California School of Medicine, Los Angeles, CA 90033, USA.

mained at that level since (Fig. 1A). Thus, both the reconstituted number of peripheral blood T cells and the elevated T cell ADA enzyme concentration have persisted since the patient's last treatment, indicating that peripheral T cells can have an unexpectedly long life-span and that gene expression from the retroviral vector has not been silenced over this period.

Patient 2, who had variable immune reactivity before enrollment, responded to the institution of lymphocyte infusions, with her peripheral T cell count rapidly increasing to levels in the high normal range (Fig 1B). Beginning with infusion 5, which included protocol modifications to partially deplete CD8 cells from the initially cultured cell population (*21*), her T cell count fell into the mid-normal range, where it persisted throughout the treatment period and for a year after the last cell infusion. In contrast to those in patient 1, ADA enzyme levels in the circulating T cells of patient 2 did not rise significantly above the small amounts seen before gene therapy treatment (~1.5 nmol/10^8 cells per minute).

The differences in final lymphocyte ADA concentration are consistent with the levels of gene transfer reached in these patients. For several months in the second protocol year during which cell infusions were not given, LASN vector sequences detected by polymerase chain reaction (PCR) maintained a stable frequency in the peripheral blood of patient 1 at a level greater than the PCR-positive control standard containing the equivalent of 0.3 vector copies/cell (Fig. 2). By contrast, although vector-containing cells were also stably detected throughout a similar period in patient 2, their level reached only a value equivalent to 0.1 to 1.0% of her circulating cells carrying the inserted ADA vector.

The principal contributor to the difference in the final frequency of LASN vector–modified T cells in patients 1 and 2 was the low gene transfer efficiency in the cells of patient 2; this was consistently only a tenth or less of what was routinely achieved in the cells from patient 1. Despite the gross differences in the final proportion of vector-containing cells reached in these two patients, both CD4 and CD8 T cell populations from each have remained consistently positive for integrated vector sequences since the first infusion through protocol day 1480 for patient 1 and through protocol day 1198 for patient 2 (Fig. 2).

To more accurately measure the proportion of vector-containing cells in patient 1, we performed quantitative Southern (DNA) hybridization analysis for vector sequence on DNA isolated from her peripheral blood T cells at different days during the course of this protocol. On protocol days 816 and 1252, which represent samples taken 109 and 545 days after the last treatment, the vector concentration was at the level of approximately one vector copy per cell (Fig. 3). Longitudinal studies of samples obtained throughout the study show that this large amount of integrated vector was reached by infusion 8 (D707) and that it has remained in this range since that time (*22*).

The use of a restriction endonuclease that cuts only once within the vector sequence does not give detectable bands (Fig. 3), indicating that the population of blood T cells at these dates is not oligoclonal with respect to integrated vector. Vector-derived mRNA was readily detected by reverse transcription (RT)–PCR at these same times (Fig. 3), confirming that vector expression persisted and was correlated with the presence of ADA enzyme activity in her circulating T cells.

To evaluate the effect of gene therapy on the immune function of these two patients in addition to its beneficial effect on T cell numbers, we performed a panel of immunologic studies both before, and at various times after, treatment. DTH skin test reactivity to common environmental and vaccine antigens tests the overall competence of the cellular immune system because a response depends on the full complement of cellular functions, not just cell proliferation or secretion of a single cytokine (Table 1). Patient 1 was anergic before our protocol treatment despite nearly 2 years of PEG-ADA treatment. Eight months after the initiation of gene therapy (protocol day 251), she had a brisk DTH response to a single intradermal skin test with tetanus toxoid. By protocol day 455, DTH responses to five of seven antigens were present, and this increased responsiveness has persisted, through day 1252.

Before the protocol, patient 2 had no positive DTH skin test (Table 1). At protocol day 501, five positive DTH skin tests were elicited, and this increased DTH reactivity had persisted when she was last tested on day 1118. She also acquired palpable lymph nodes and visible tonsils during the period of protocol treatment.

To corroborate the improved immune function indicated by these DTH tests, we evaluated the capacity of peripheral T cells from our patients to produce interleukin-2 (IL-2) or to kill antigenic target cells in vitro. In several patients treated with PEG-ADA, in vitro T cell proliferative responses to mitogens may normalize, whereas responses to specific antigens are less improved (*7–10*). During PEG-ADA treatment before gene therapy, T cells from patient 1 produced IL-2 in response to stimulation with

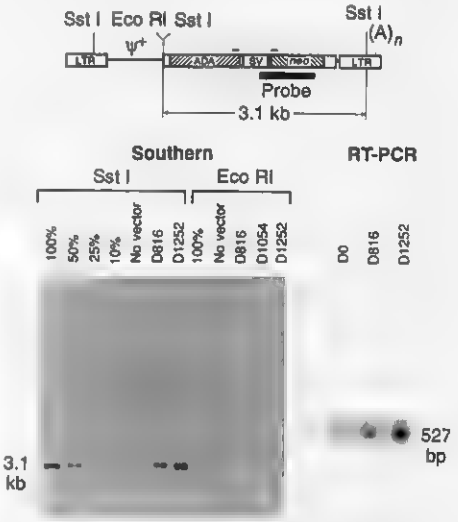

Fig. 3. Quantitative Southern hybridization analysis of DNA prepared from the blood mononuclear cells of patient 1 on protocol days (D) 816 and 1252 (*28*). DNA digested with Sst I should yield a single restriction fragment of 3.1 kb containing both the vector *neo* and ADA genes. Eco RI cuts only once within the vector sequence, and therefore a detectable band would indicate that a predominant clone with a single unique vector integration site was present in that blood sample. None was detected. Polyadenylated mRNA was extracted from the patient cells on days 0, 816, and 1252 and analyzed for vector message by RT-PCR (*29*). The primer locations used are indicated as short solid lines above the vector diagram. SV, SV40 early promoter; $(A)_n$, polyadenylation site; Ψ, extended retrovirus packaging signal. Hatched regions indicate protein coding regions.

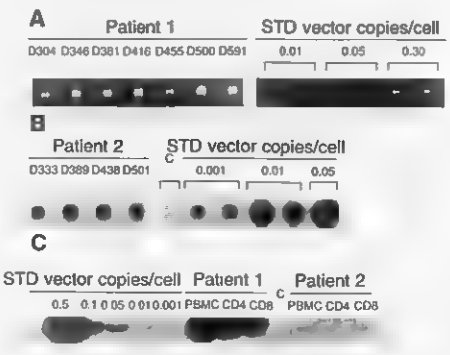

Fig. 2. PCR evaluation of the frequency of LASN vector–positive cells in the blood of patients 1 and 2 at various protocol days. (**A**) Cells from patient 1 for protocol days (D) 304 to 591 (see Fig. 1A). PCR analysis was performed as described (*26*) in an ethidium-stained gel. (**B**) Cells from patient 2 for protocol days (D) 333 to 501 (see Fig. 1B). PCR products were probed with ^{32}P-labeled *neo* gene as described (*26*). (**C**) Purified $CD4^+$ and $CD8^+$ cell subpopulations from patient 1 (D1480) and patient 2 (D1198) prepared by separation of peripheral blood mononuclear cells (PBMCs) by fluorescence-activated cell sorting (FACS). The purity of the separated T cell subpopulations from which DNA was extracted exceeded 98%, as confirmed by FACS analysis. Direct PCR with [^{32}P]deoxycytosine triphosphate was performed as described (*27*). Standards (STD) were prepared from DNA obtained from cell mixtures of a known proportion of LASN-transduced cells containing a single vector insert mixed with vector-negative cells. C, vector-negative control cells.

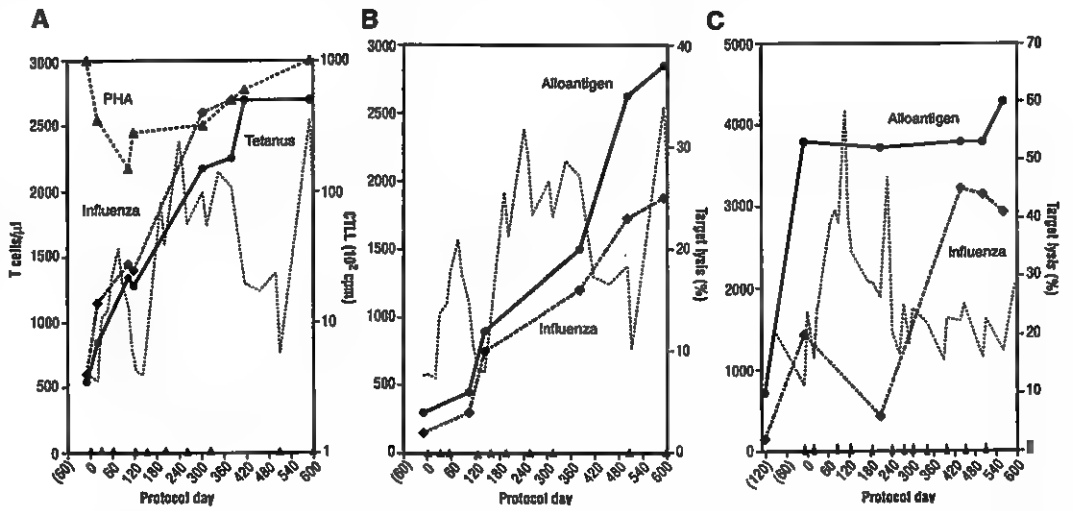

Fig. 4. Evaluation of the in vitro cellular immune responses of blood T cells from patients 1 and 2 at various times before and during the gene therapy trial. At least two normal subjects were included concurrently in each assay, and only those in which the controls responded appropriately are included here. **(A)** Production of IL-2 by cultured cells from patient 1 after stimulation with the mitogen PHA and with the specific antigens tetanus toxoid and influenza A virus as described (*30*). IL-2 was quantitated by bioassay measuring the proliferation of the IL-2–dependent T cell line CTLL at a 1:2 dilution of the lymphocyte culture supernatant. The fine dashed line indicates the patient's T cell count for reference. Solid triangles along the base line indicate the dates of cell infusion. **(B)** In vitro killing of a ^{51}Cr-labeled, influenza A–infected autologous B cell line and a ^{51}Cr-labeled allogeneic target B cell line by blood T cells from patient 1 as described (*31*). Lysis (as percent specific isotope release during a 6-hour incubation of effector and target cells at a ratio of 60:1) was measured after in vitro pre-stimulation for 7 days. Solid triangles along the base line indicate the dates of cell infusion. **(C)** In vitro killing of a ^{51}Cr-labeled, influenza A–infected autologous B cell and a ^{51}Cr-labeled allogeneic target B cell line by blood T cells from patient 2 as described above.

the mitogen phytohemagglutinin (PHA) (Fig. 4A) but were unable to produce IL-2 in response to stimulation with influenza A virus or tetanus toxoid, despite repeated immunization with these antigens. Over the first months of gene therapy, IL-2 production improved and became normal after 1 year (Fig. 4A). Again before gene therapy, patient 1's T cells failed to show significant cytolytic reactivity against either allogeneic cells or influenza A–infected target cells. Almost mirroring the steady increase in IL-2 production, she acquired normal in vitro cytolytic T cell responses to these antigens, reaching normal values in her second year of treatment. (Fig. 4B).

The results of these cytolytic assays for patient 2 are shown in Fig. 4C. Tests done 120 days before the beginning of gene therapy also showed impaired responses. However, cells that were obtained at the time of the first gene therapy infusion, cryopreserved, and subsequently tested some months later showed a normal cytolytic response to allogeneic cells. After a year on gene therapy, cytolytic T cell activity against influenza also became normal.

To evaluate the effects of our treatment on humoral immune function in these patients, we measured antibody responses to several antigens. Despite their PEG-ADA treatment, both patients 1 and 2 had only low or borderline titers of isohemagglutinins on repeated testing before gene therapy. Each patient showed significant elevations in the levels of these antibodies within 90 to 115 days of beginning treatment with gene-modified cells (Table 1). Isohemagglutinins are antibodies that react with group A and B red blood cell antigens and occur spontaneously as a result of environmental exposure to cross-reacting antigens. Isohemagglutinin responses are, therefore, less dependent on the timing of previous immunizations than are responses to common vaccine antigens. After gene therapy, each patient also had improvement in antibody responses to vaccines to *Hemophilus influenzae* B (HIB) and tetanus toxoid (Fig. 5). With enzyme therapy alone, peripheral lymphocytes from each patient were unable to produce immunoglobulin M (IgM) in vitro after stimulation with pokeweed mitogen (PWM), but made robust responses after a year on the gene therapy protocol (Fig. 5A). Immunoglobulin production to PWM depends on T cells; these results further confirm the reconstitution of T cell function associated with gene therapy.

The effects of this treatment on the clinical well-being of these patients is more difficult to quantitate. Patient 1, who had been kept in relative isolation in her home for her first 4 years, was enrolled in public kindergarten after 1 year on the protocol and has missed no more school because of infectious disease than her classmates or siblings. She has grown normally in height and weight and is considered to be normal by her parents. Patient 2 was regularly attending public school while receiving PEG-ADA treatment alone and has continued to do well clinically. Chronic sinusitis and headaches, which had been a recurring problem for several years, cleared completely a few months after initiation of the protocol.

This trial of retroviral-mediated gene transfer shows that the survival of reinfused transduced peripheral blood T cells is prolonged in vivo; the erroneous assumption that T cells would not have such long-term survival was often cited as a potential problem with this treatment strategy. Patient 1 has had a normal total peripheral T cell count since the last cell infusion, and the proportion of her circulating T cells carrying vector DNA has remained stable over that period. Further, expression of the ADA transgene under the influence of the retroviral long terminal repeat (LTR) promoter has persisted for a long period in vivo without obvious extinction. There have been swings in the level of ADA enzyme in her peripheral lymphocytes throughout the period of observation, but the level of blood ADA enzyme activity at 4 years (protocol day 1480) is equivalent to that found immediately after the last cell infusion 2 years earlier (Fig. 1A). Although the data have not yet been completely analyzed, blood obtained after 5 years showed continuation of this trend with, again, a normal T lymphocyte count and an equivalent ADA level.

The mechanism by which our treatment aided immune reconstitution in patient 2 is less clear. The responses of patient 2 to some in vitro immunologic tests were variable before beginning our treatment protocol, ranging from little or no detectable response to nearly normal responses on the blood sample from the day gene therapy began. This patient produced a normal antibody response to immunization with bacteriophage φX174 about a year before beginning gene therapy (8). Although we have shown several examples of depressed cellular and humoral immune responses that strongly improved after gene therapy, this highly variable immune reactivity while patient 2 was on PEG-ADA therapy alone complicates interpretation of the contribution of our therapy. There was a temporal relation between initiation of gene therapy and a normalized peripheral T cell count,

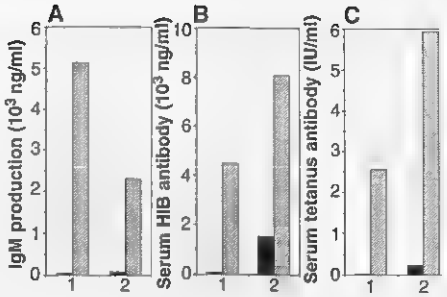

Fig. 5. Humoral immune function of patients 1 and 2 before (solid bars) and after (hatched bars) gene therapy. (**A**) IgM production by the patient's peripheral blood mononuclear cells in cultures stimulated with the T cell–dependent polyclonal activator PWM performed as described (*32*). "Before" samples were from D(−9). Follow-up cultures were at D500 (patient 1) and D560 (patient 2). In each case, the patient's cells stimulated with the T cell–independent B cell stimulant EBV (*33*) produced normal amounts of IgM (not shown), indicating intact B cell function before and after gene therapy, as expected. At least two normal subjects were included concurrently in each assay, and only those in which the controls responded appropriately are included here. (**B**) Serum antibody response to *Hemophilus influenzae* B. Patient 1 had failed to respond to two immunizations while on PEG-ADA alone [D(−9) shown]. Her response at protocol D591 is shown, after immunization. Patient 2 had some HIB-specific antibodies present before therapy [D(−122)], whose amounts increased without additional immunization during the protocol (D560). (**C**) Serum tetanus antibody. Patient 1 had negligible response to five separate tetanus immunizations before gene therapy [D(−48) shown] but responded briskly at D731, 24 days after re-immunization. Serum titers for patient 2 are shown for D(−9), 140 days after immunization while on PEG-ADA alone, and after receiving gene therapy (D592), 32 days after a booster tetanus immunization.

improved DTH, appearance of tonsils and palpable lymph nodes, normalized isohemagglutinin response, and improved PWM response, as well as other factors. In view of the relatively low level of ADA gene transfer achieved in this patient, the potential contribution of the infusions of the culture-activated T cells to the patient's response must also be considered. Perhaps ex vivo T cell activation somehow bypassed a differentiation block that PEG-ADA alone was unable to relieve. Despite the low final percentage gene transfer achieved, a 1% level of ADA gene–corrected cells could represent 10^9 to 10^{10} ADA-expressing T cells distributed throughout the body that could readily contribute to immune improvement.

Since the beginning of the trial, the dose of PEG-ADA enzyme given to each of our patients has been decreased by more than half (patient 1, 14 U/kg/week; patient 2, 10 U/kg/week), during which time their immune function has improved. By contrast, worsened immune function has been seen in other ADA$^-$ SCID patients when their dose of enzyme has been similarly reduced (*10*, *23*). We do not want to expose these patients to the potential risk of recurrent immunodeficiency by completely stopping PEG-ADA enzyme treatment until we have better information about the quality and duration of the immune improvement achieved by this first-generation gene therapy trial. The role of continued exogenous enzyme treatment will be clarified here or in companion studies attempting stem cell gene correction (*24*).

The safety of retroviral-mediated gene transfer has been a central concern. At least in the short and intermediate term, no problems have appeared in any clinical trial using these vectors. In the longer term, the theoretical potential for retroviral vectors to cause insertional mutagenesis remains the primary concern. To date, there has been no indication that malignancy associated with this process will be a complication of retroviral-mediated gene transfer.

Our trial here has demonstrated the potential efficacy of using gene-corrected autologous cells for treatment of children with ADA$^-$ SCID. Eleven children with this disease have been enrolled in various gene therapy protocols, each using different strategies and retroviral vector designs and focusing on different target cell populations. The experience gained from these approaches should provide guidance for gene therapy as a treatment for this disorder as well as for a larger array of inherited and acquired diseases.

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17. Peripheral T cells from the patients were collected by apheresis, isolated by density gradient centrifugation, washed extensively, and then cultured in 24-well culture plates in medium supplemented with 100 to 1000 IU/ml of recombinant IL-2 and 10 ng/ml of OKT3 to stimulate T cell proliferation. After 24 hours, half the medium was removed and replaced with supernatant containing the LASN retroviral vector supplemented with IL-2 and protamine (10 μg/ml) to give an initial multiplicity of infection of 1. The LASN vector contains the human ADA cDNA under the transcriptional control of the promoter-enhancer in the retroviral LTR and a neomycin phosphotransferase gene (*neo*) controlled by an internal SV40 promoter [R. A. Hock, A. D. Miller, W. R. A. Osborne, *Blood* **74**, 876 (1989)] LASN was packaged with PA317 amphotropic retrovirus packaging cells (*2*). The LASN vector preparation, manufactured under good manufacturing practices by Genetic Therapy, Gaithersburg, MD, had a titer of 1×10^5 to 3×10^5. The cells were returned to the incubator and the transduction process repeated, with the addition of fresh retroviral supernatant and IL-2 twice daily for a total of three to five additions of vector. The cultured cells were transferred to gas-permeable culture bags at the conclusion of the transduction process. The proliferating T cell cultures were observed daily, split, and fed as necessary with periodic samples tested for viability and microbial contamination Gene transfer efficiency was variable from treatment to treatment and patient to patient, ranging from 1 to 10% for patient 1 and 0.1 to 1% for patient 2. On days 9 to 12, the cultured cells were washed extensively with saline containing 0.5% human albumin and were then infused into the patient over a period of about 1 hour. During the 9 to 12 days of culture, the cell populations had expanded 17- to 135-fold Preliminary studies testing the T cell receptor β gene repertoire showed that T cell cultures remained polyclonal for at least 3 weeks under these culture conditions. The culture period used in the clinical trial was held to half this time period to ensure a polyclonal T cell repertoire in the infused cell population.
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20. The protocol was reviewed and approved by the Clinical Research Subpanels of the NCI and NHLBI, the NCI Cancer Treatment and Evaluation Program (CTEP), the NIH Biosafety Committee, the Human Gene Therapy Subcommittee, the Recombinant DNA Advisory Committee, the Director of NIH, and the U.S. Food and Drug Administration. Informed consent was obtained from the parents of each patient.
21. Beginning with culture 9 for patient 1 and culture 5 for patient 2, the patients' lymphocyte populations obtained by apheresis were fractionated by adherence to flasks coated with CD8 monoclonal antibodies (Applied Immune Sciences) following the manufacturer's instructions. This protocol modification for CD8 depletion was introduced because both patients were developing a progressively inverted CD4-CD8 ratio. This effect was apparently the result of preferential growth of $CD8^+$ cells during the last 4 to 5 days of culture and the subsequent persistence of these infused $CD8^+$ cells in the circulation. Consequently, each subsequent apheresis sampled the recently increased number of $CD8^+$ cells, and thus the skewing of the ratio of CD4 to CD8 cells became compounded with each additional treatment. By partially depleting the apheresis sample of $CD8^+$ cells by an immunoaffinity selection process, the later treatments for each patient consisted of cells with a more balanced phenotype. The perturbation in normal CD4-CD8 cell proportions did not have detectable untoward effects for either patient.
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25. The ADA enzyme activity assay was performed in duplicate as described (*13*). Positive control cells were obtained from healthy normal donors and had a mean of 82 U (normal range, 66 to 102 U). Duplicate samples were run in the presence of the ADA enzyme inhibitor EHNA (30 μM). Specific ADA activity was calculated as total adenosine deaminating activity minus EHNA-resistant activity. EHNA-resistant activity represents metabolic activity of a nonspecific aminohydrolase present in human cells.
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28. Southern hybridization analysis for LASN vector consisted of the following: 10 μg of DNA was digested with Sst I and hybridized with a 728-bp Nco I fragment from LASN corresponding to the SV40 promoter and *neo* gene. DNA from K562-LASN cells served as a positive control.
29. RT-PCR analysis for LASN vector transcripts was as follows: 3 μg of polyadenylated RNA was treated with deoxyribonuclease and reverse-transcribed. The cDNA (0.3 μg) was amplified with LASN vector–specific primers in a 30-cycle PCR reaction. The oligonucleotides 5′-CAGCCTCTGCAGGGCAGAAC-3′ (corresponding to the 3′ end of the ADA gene in LASN) and 5′-GCCCAGTCATAGCCGAATAG-3′ (complementary to 5′ end of the *neo* gene in LASN) were used as primers. After electrophoresis and blotting, the sequences were hybridized with a 527-bp probe corresponding to the entire length of the predicted PCR product.
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36. The authors acknowledge superb technical and nursing support by C. Able, K. Snitzer, A. Roop, M. Brown, F. May, R. Gutieriez, M. Yu, H. Goetzman, C. Wannebo, B. Sink, and L. Top. In addition, we thank R. Sorensen for providing the initial patient blood samples, Applied Immune Sciences for CELLector T-150 flasks, and Genetic Therapy for clinical-grade LASN vector. Finally, we also thank our earlier collaborators D. B. Kohn, E. Gilboa, P. W. Kantoff, M. A. Eglitis, R. Moen, K. Cornetta, A. Gillio, R. J. O'Reilly, and C. Bordignon for their contributions that helped prepare the way for this clinical trial.

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Physical Map and Organization of *Arabidopsis thaliana* Chromosome 4

Renate Schmidt,* Joanne West, Karina Love, Zoë Lenehan, Clare Lister, Helen Thompson, David Bouchez, Caroline Dean†

A physical map of *Arabidopsis thaliana* chromosome 4 was constructed in yeast artificial chromosome clones and used to analyze the organization of the chromosome. Mapping of the nucleolar organizing region and the centromere integrated the physical and cytogenetic maps. Detailed comparison of physical with genetic distances showed that the frequency of recombination varied substantially, with relative hot and cold spots occurring along the whole chromosome. Eight repeated DNA sequence families were found in a complex arrangement across the centromeric region and nowhere else on the chromosome.

Arabidopsis thaliana has been adopted as a model organism for the analysis of complex plant processes by means of molecular genetic techniques (*1*). The increase in map-based cloning experiments makes the generation of a complete physical map of the *Arabidopsis* genome a high priority. In addition, the availability of such a map would enable the organization of the chromosome to be studied in more detail. Little is known about the organization of plant chromosomes, but the general picture is that of chromosomes carrying large numbers of dispersed [often retrotransposons (*2*)] and tandemly repeated DNA sequences (*3*). The relatively small (100 Mb) *Arabidopsis* genome has a much smaller number of repeated DNA sequences than do most other plant species; its five chromosomes contain ~10% highly repetitive and ~10% moderately repetitive DNA (*4*). The dispersion of most of these sequences among the low-copy DNA is unknown.

We discuss here a physical map, which we have presented on the World Wide Web (WWW) at URL: http://nasc.nott.ac.uk/JIC-contigs/JIC-contigs.html, of *Arabidopsis* chromosome 4, one of the two chromosomes carrying nucleolus organizing regions. The construction of this map allowed us to analyze the frequency of recombination along the whole chromosome, the integration of the physical with the cytogenetic map, the interspersion pattern of repeated and low-copy DNA sequences over the whole chromosome, and the arrangement of repeated DNA sequences over the centromeric region.

We generated the physical map by hybridizing probes to four yeast artificial chromosome (YAC) libraries (*5*), using colony hybridization experiments (*6*). The probes consisted of 112 markers genetically mapped to chromosome 4, 20 previously unmapped genes, random genomic fragments and sequences flanking transposable elements, and the 180–base pair (bp) repetitive element carried in pAL1 (*7*). Southern (DNA) blot analysis of YAC clones confirmed the colony hybridization results and revealed common restriction fragments in the different YAC clones hybridizing to a given marker. This demonstrated overlap between the inserts of the YAC clones. On the basis of these results, the YAC clones could be placed into 14 YAC contigs with a high degree of redundant YAC cover, ensuring an accurate map despite the presence of chimeric clones in the YAC libraries.

We generated YAC end fragments, using either inverse polymerase chain reaction (IPCR) or plasmid rescue (*8*), from YAC clones lying near the ends of each of the 14 contigs. The fragments were hybridized to Southern blots of YAC clones from adjacent contigs. In addition, YACs, as well as some of the end fragments generated by IPCR, were used to identify clones from a cosmid library of the Columbia ecotype (*9*). The cosmids were then used as new markers on the YAC libraries. These experiments reduced the number of contigs to four. In all but two instances, the end fragments revealed that the contigs were already overlapping. Experiments aimed at closing the last three gaps have been attempt-

R. Schmidt, J. West, K. Love, Z. Lenehan, C. Lister, H. Thompson, C. Dean, Department of Molecular Genetics, Biotechnology and Biological Sciences Research Council, John Innes Centre, Colney, Norwich NR4 7UH, UK. D. Bouchez, Laboratoire de Biologie Cellulaire, Institut National de la Recherche Agronomique, 78026 Versailles Cedex, France.

*Present address: Max-Delbrueck Laboratory, Carl-von-Linne-Weg 10, D50829, Cologne, Germany.
†To whom correspondence should be addressed.

ed but so far have been unsuccessful. Two of the gaps map to the centromeric region, where repetitive sequences severely limit walking experiments. Sparse YAC coverage as well as chimeric YAC clones have made the walking experiments in the area between contigs III and IV particularly difficult. Neither the generation of end fragments nor the hybridization of YAC clones to cosmid libraries has detected an overlap or extended the contigs. The physical map of 374 YAC clones is represented in a format in which each marker hybridized to the YAC libraries is shown a unit length away from the next; thus, the sizes of the YAC clones only reflect their marker content, not their physical size. Multiple YAC coverage has been achieved for the majority of the chromosome. Only 12 of the 148 links are spanned by one YAC clone.

We determined the physical distance between 77 markers by integrating several types of data: the size of the YAC inserts, the minimum size of a YAC clone spanning two markers, and whether each YAC clone ended within or spanned a complete marker (*10*). Because of the redundancy of YAC clones covering each interval and the inclusion of small as well as large insert YAC clones, we estimate the error for these distances to be only ±10%. The total size of the four YAC contigs is approximately 17 Mb, and they cover a minimum of 82.5 centimorgans (cM), or 90% of the chromosome 4 genetic map (Fig. 1).

A comparison of genetic with physical distance over 75 intervals spanning the whole chromosome is shown in Fig. 1. The genetic distances were derived from up to 100 lines of a single recombinant inbred mapping population (*11*) and so can be compared directly. The ratio of physical to genetic distance between markers varied significantly along the length of the chromosome, with the average value for the four contigs being 185 kb/cM. Relative cold spots (>550 kb/cM) were distributed throughout the chromosome, in intervals close to the centromere (mi233 to g2616 to m506), but also in intervals in other chromosomal locations (g3883 to JGB9 and 06455 to g3088) (Fig. 1). Relative hot spots (30 to 50 kb/cM) (mi51 to mi204, m518 to BIO216, mi128 to g6837, and m214 to ap2) were also distributed throughout the chromosome (Fig. 2). There was no distinct association of higher recombination frequencies with certain chromosomal regions. This is different from the situation reported for yeast chromosome 3 (*12*), in which recombination was lowest close to the centromere and highest midway down each arm. It also contrasts with the picture emerging for tomato and wheat chromosomes (*13*), in which recombination is strongly suppressed over large intervals at the centromere, with maximum recombination occurring in the proximal regions.

In order to integrate the physical and cytogenetic maps, it was necessary to place the centromere and the nucleolar organizing region (NOR) that maps to chromosome 4 (*14*, *15*) on the physical map. An end fragment from YAC clone CIC5C11 (CIC5C11LE) was found to contain ribosomal DNA (rDNA) sequences. The YAC also hybridized to markers BIO217 and mi51. Southern blot analysis of two additional YAC clones (yUP2A9 and yUP7C3) hybridizing to these markers showed that they also contained rDNA sequences. These results positioned the NOR locus distal to marker BIO217 and all known chromosome 4 markers (Fig. 1). We did not attempt to obtain YAC clones covering the NOR locus because of the complication of the instability of YAC clones carrying rDNA sequences (*16*). NOR 4 has recently been genetically mapped as the most terminal marker on chromosome 4; it is in close physical proximity to the telomere (*17*). Thus, the NOR locus defines one end of the physical map of chromosome 4.

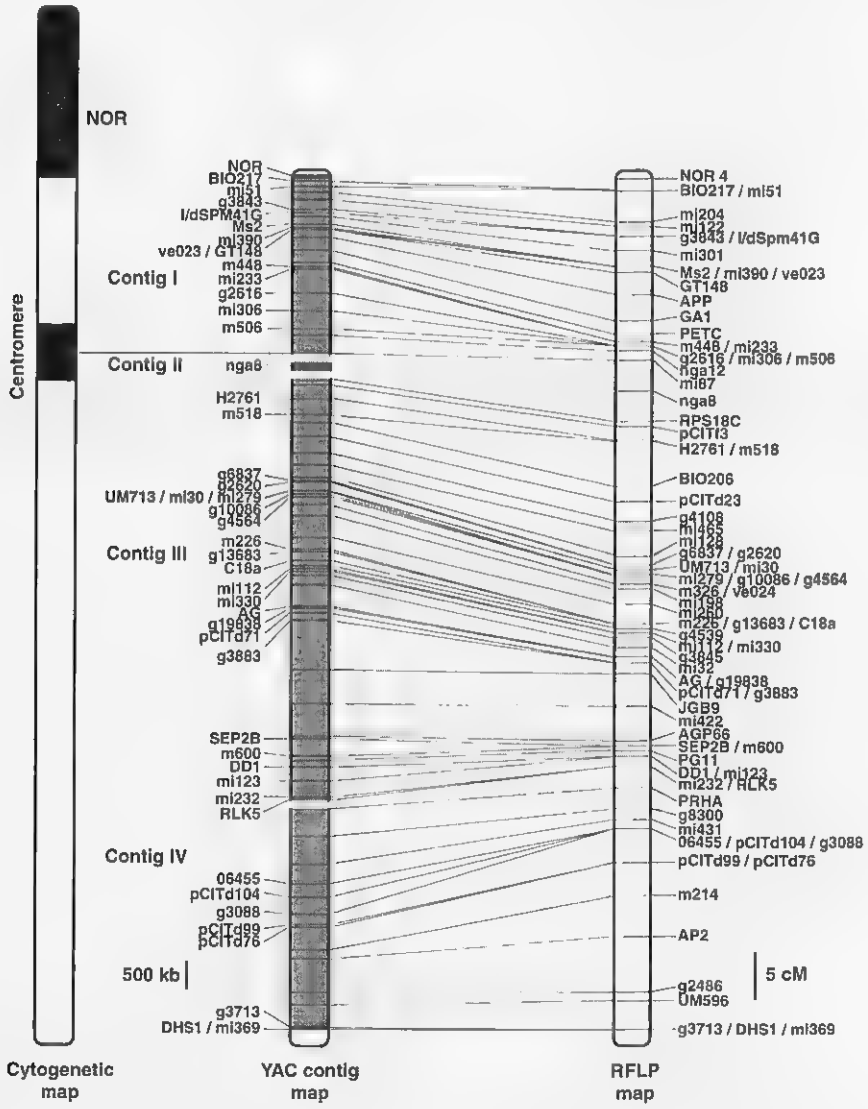

Fig. 1. The alignment of the cytogenetic, YAC contig, and RFLP maps of chromosome 4. The schematic drawing of the cytogenetic map [adapted from (*26*)] approximately reflects the actual physical sizes of the various chromosomal regions. The RFLP map shows positions for 80 loci. These loci were mapped on up to 100 recombinant inbred lines derived from a cross between the ecotypes Landsberg *erecta* and Columbia (*11*). The most likely marker order as determined empirically with the MAPMAKER program (version 3.0) (*27*) differed in four places from the order as derived from the physical map. These intervals and the log of the likelihood ratio of the most likely order predicted by MAPMAKER to the order as determined by physical mapping are as follows: nga12 to mi87 (0.57); m226 to g3845 (4.23); ag to g3883 (0.21); and agp66 to mi232 (0.73). Because the physical ordering of these markers was unambiguous, map distances were calculated [with the use of the Kosambi mapping function (*28*)] by MAPMAKER on the basis of the order determined from the physical map. The relative positions of all loci on the YAC contig and genetic map are indicated by lines across the chromosomes. Only markers that cosegregate with adjacent markers on the RFLP map are shown next to the YAC contig map. The four YAC contigs are shown as gray boxes. Contig II and the gaps between the contigs are not drawn to scale.

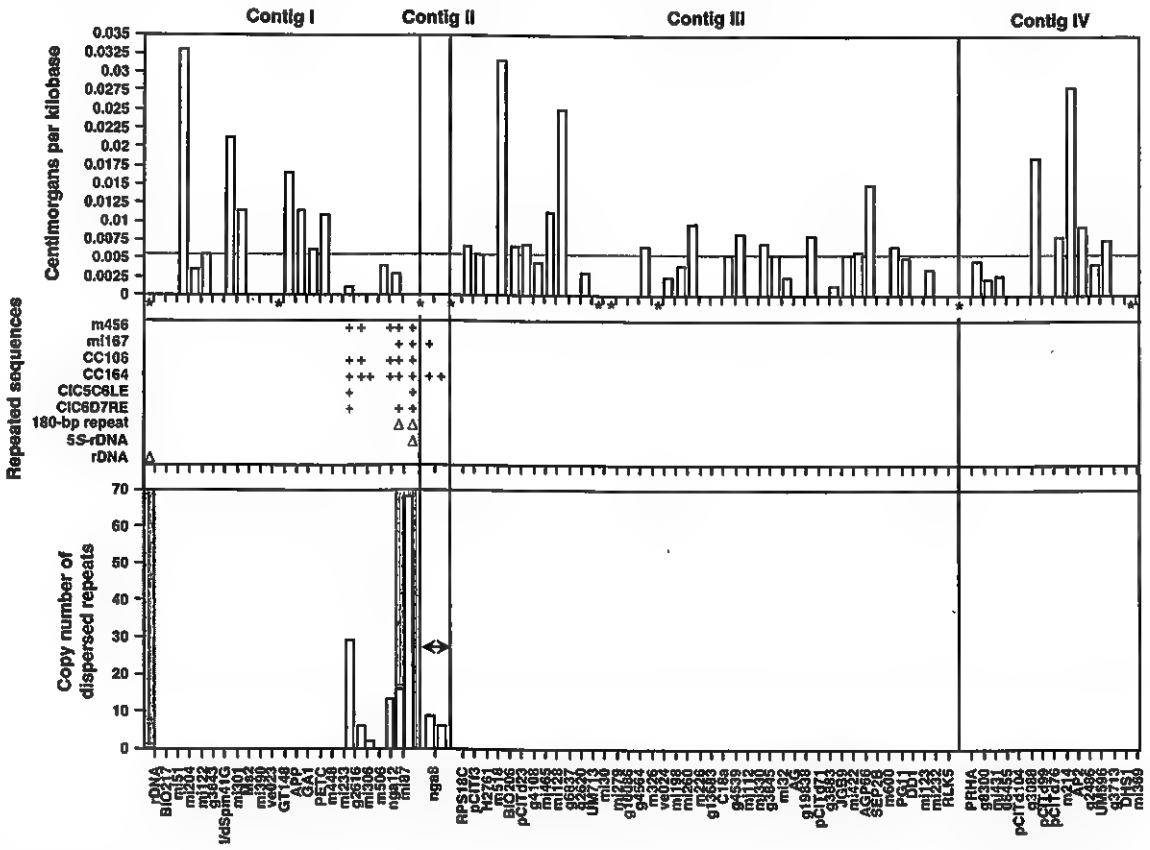

Fig. 2. Distribution of recombination hot spots and nine families of repetitive elements on chromosome 4. All genetically mapped markers are shown a unit length away from each other at the bottom of the figure. Vertical lines represent the boundaries of the YAC contigs. The top panel shows the ratio of centimorgans to kilobases for the intervals between markers. The average value for chromosome 4 is indicated by a horizontal line. Intervals for which the physical distance between markers could not be established, either because markers could not be separated or because of gaps between contigs, are marked by asterisks. The middle panel shows the presence of several classes of repeated sequences along the length of the chromosome. All sequences noted were analyzed across all chromosomal intervals noted. Dispersed repeat sequences are indicated by plus signs; tandemly repeated sequences are indicated by triangles. The bottom panel shows the cumulative copy number of the six dispersed repeated sequence families (indicated by plus signs in the middle panel) in each interval. To determine the copy number, we hybridized Southern blots carrying YAC clone DNA–digested by Eco RI–Bam HI to the various dispersed repeats, and the number of hybridizing restriction fragments was determined for each of the repeated sequences. The presence of tandemly repeated sequences is indicated as gray boxes. Double-headed arrow indicates that the orientation of contig II has not been determined.

Due to the lack of suitable genetic stocks, the centromere had not been mapped relative to phenotypic or restriction fragment length polymorphism (RFLP) markers. The centromeric region of chromosome 4 could be positioned by identification of YAC clones that hybridized to the tandemly repeated 180-bp sequence contained in plasmid pAL1 (*18*). This family of tandemly repeated DNA sequences is present in arrays of >50 kb, makes up 1 to 1.6% of the *Arabidopsis* genome, and has been shown to co-localize with the heterochromatin surrounding the centromeres of all five *Arabidopsis* chromosomes (*14*, *18*, *19*). CIC YAC clones hybridizing to pAL1 as well as to two markers (mi87 and nga12) mapping to chromosome 4 were identified. Southern blot analysis of DNA from these YAC clones digested with several restriction enzymes revealed the presence of two different hybridization patterns corresponding to two 180-bp repeat loci (each carrying a large number of tandemly repeated copies) flanking mi87 (Fig. 3). Maluszynska and Heslop-Harrison (*14*) found in in situ hybridization experiments that the 180-bp tandemly repeated sequence hybridized equally to both sides of the centromeres on all five chromosome pairs. Taken together, one could conclude that the YAC contig shown in Fig. 3 covers at least the core of the centromere.

The mapping of NOR 4 and the centromere allowed the integration of the physical and cytogenetic maps (Fig. 1). YAC contig I covers the short arm of chromosome 4, with most of the genetic map residing on the long arm and being contained in YAC contigs II, III, and IV.

The availability of YAC clones covering most of chromosome 4 enabled the distribution of low- and high-copy sequences to be examined. Pruitt and Meyerowitz (*20*) analyzed a random set of lambda clones for the presence of repetitive DNA. They showed that the average single-copy sequence length would be 125 kb if the repeated DNA sequences were randomly distributed. Thus, the sequence interspersion pattern of *Arabidopsis* is extremely long relative to that of other plant genomes. Southern blots carrying a representative set of YAC clones covering the chromosome 4 contigs were hybridized with nine different repeated DNA sequences. As described above, rDNA sequences were localized on YAC clones at the top of the map and the 180-bp repeated sequence was localized on YAC clones at the bottom of contig I (Fig. 2). They were not detected elsewhere on the chromosome. 5S-rDNA sequences (*21*) hybridized to a number of YAC clones that contained the 180-bp repeated sequence as well as marker mi87. Thus, one of the 5S-rDNA loci in *Arabidopsis* (*21*) maps to the centromeric region of chromosome 4 (Figs. 2 and 3).

At least six other repeated DNA sequences were identified during the course of this work. Two RFLP markers, m456 and mi167, were found to hybridize at high stringency to ~ 50 to 100 fragments in *Arabidopsis* DNA. In addition, two cosmid clones carrying Columbia DNA, CC106 and CC164, were identified that hybridized strongly to total *Arabidopsis* DNA but did not cross-hybridize with any previously characterized repetitive sequence. They hybridized to ~ 300 and 150 *Arabidopsis* fragments, respectively. Sequence analysis of mi167 and subclones from CC106 and CC164 revealed no significant homology to sequences in all available databases (*22*). We have not yet established how many different families of repeated DNA sequences are carried on m456, mi167, CC106, and CC164. Two other repeated DNA sequence families were identified as end fragments from YAC clones CIC5C6LE and CIC6D7RE.

The six clones were hybridized to the

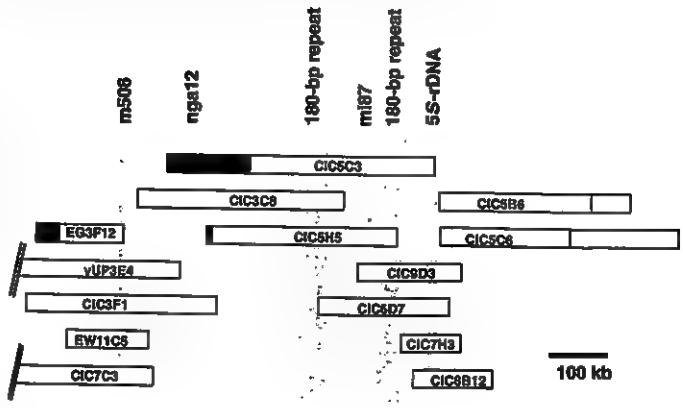

Fig. 3. Contig covering the centromeric region, showing the YAC clones covering this part of the chromosome. The sizes of all the YAC inserts are drawn to scale. The arrangement of the YAC clones shown is consistent with data on hybridization to the markers shown at the top and to the dispersed repetitive elements shown in Fig. 2, as well as with data from a limited number of chromosome walking experiments (*29*). The sizes shown for the 180-bp repeat (pAL1) and 5*S*-rDNA are not drawn to scale. Noncontiguous sequences in known chimeric clones are shown as black boxes. Slashes at left indicate that only part of these clones is shown. Instability was observed in some of the YAC clones containing the tandemly repeated 180-bp Hind III (pAL1) sequence (*16*).

Southern blots containing the representative set of YAC clones covering the chromosome 4 contigs. They all hybridized to multiple fragments on YAC clones mapping to the bottom of contig I and to contig II (Fig. 2). The copy number of all the repeated sequences was largest in the YAC clones closest to the 180-bp repeated sequence loci. Unlike the 180-bp repeated sequences and 5*S*-rDNA sequences, these dispersed repeats were also found in several positions up to 1.5 Mb from the centromere in contig I and in YAC contig II (Fig. 2). Long stretches of complex repeated DNA sequences are required for centromere function in *Shizosaccharomyces pombe* (*23*) and *Drosophila* (*24*), and an α-satellite DNA has been implicated in centromere function in human chromosomes (*25*). A functional analysis will be required to establish whether the tandem 180-bp repeated sequence and the dispersed repeats contribute to centromere function on chromosome 4. At the relatively high stringency used in the experiments, YAC clones mapping to the rest of chromosome 4 were devoid of sequences that hybridize to these clones (Fig. 2).

In conclusion, the size of the four YAC contigs shown in Fig. 1 is ~17 Mb. If the NOR carrying the tandemly repeated rDNA units is 3.5 Mb (*17*) and the gaps are ~1.0 Mb, then the total size of chromosome 4 would be on the order of ~21.5 Mb. The eight repeated sequence families analyzed were clustered around the centromere and most likely constitute the flanking heterochromatin. This finding, along with the relative lack of repeated DNA sequences found in the chromosome walking experiments, suggests that the majority of both arms (except for the NOR locus) is composed predominantly of medium- and low-copy DNA. Recombination frequency varied along the chromosome, with some suppression being detected around the centromere, in the region carrying most of the repeated DNA sequences (Fig. 2). However, other chromosomal regions showing equally low recombination frequencies were not associated with these particular repeated sequence families (Fig. 2). The extreme localization of the repetitive DNA distinguishes the organization of the *Arabidopsis* chromosomes from that so far described for other plant chromosomes.

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Serial Analysis of Gene Expression

Victor E. Velculescu, Lin Zhang, Bert Vogelstein, Kenneth W. Kinzler*

The characteristics of an organism are determined by the genes expressed within it. A method was developed, called serial analysis of gene expression (SAGE), that allows the quantitative and simultaneous analysis of a large number of transcripts. To demonstrate this strategy, short diagnostic sequence tags were isolated from pancreas, concatenated, and cloned. Manual sequencing of 1000 tags revealed a gene expression pattern characteristic of pancreatic function. New pancreatic transcripts corresponding to novel tags were identified. SAGE should provide a broadly applicable means for the quantitative cataloging and comparison of expressed genes in a variety of normal, developmental, and disease states.

Determination of the genomic sequence of higher organisms, including humans, is now a real and attainable goal. However, this analysis represents only one level of genetic complexity. The ordered and timely expression of this information represents another level of complexity equally important to the definition and biology of the organism. Techniques based on complementary DNA (cDNA) subtraction or differential display can be quite useful for comparing gene expression differences between two cell types (*1*), but provide only a partial picture, with no direct information about abundance. The expressed sequence tag (EST) approach is a valuable tool for gene discovery (*2*), but like RNA blotting, ribonuclease (RNase) protection, and reverse transcriptase–polymerase chain reaction (RT-PCR) analysis (*3*), it evaluates only a limited number of genes at a time. Here we describe the serial analysis of gene expression (SAGE), a technique that allows a rapid, detailed analysis of thousands of transcripts.

SAGE is based on two principles. First, a short nucleotide sequence tag [9 to 10 base pairs (bp)] contains sufficient information to uniquely identify a transcript, provided it is isolated from a defined position within the transcript. For example, a sequence as short as 9 bp can distinguish 262,144 transcripts (4^9) given a random nucleotide distribution at the tag site, whereas current estimates suggest that even the human genome encodes only about 80,000 transcripts (*4*). Second, concatenation of short sequence tags allows the efficient analysis of transcripts in a serial manner by the sequencing of multiple tags within a single clone. As with serial communication by computers, wherein information is transmitted as a continuous string of data, serial analysis of the sequence tags requires a means to establish the register and boundaries of each tag.

Figure 1 shows how these principles were implemented for the analysis of mRNA expression. Double-stranded cDNA was synthesized from mRNA by means of a biotinylated oligo(dT) primer. The cDNA was then cleaved with a restriction endonuclease (anchoring enzyme) that would be expected to cleave most transcripts at least once. Typically, restriction endonucleases with 4-bp recognition sites were used for this purpose because they cleave every 256 bp (4^4) on average, whereas most transcripts are considerably larger. The most 3′ portion of the cleaved cDNA was then isolated by binding to streptavidin beads. This process provides a unique site on each transcript that corresponds to the restriction site located closest to the polyadenylate [poly(A)] tail. The cDNA was then divided in half and ligated via the anchoring restriction site to one of two linkers containing a type IIS restriction site (tagging enzyme). Type IIS restriction endonucleases cleave at a defined distance up to 20 bp away from their asymmetric recognition sites (*5*). The linkers are designed so that cleavage of the ligation products with the tagging enzyme results in release of the linker with a short piece of the cDNA.

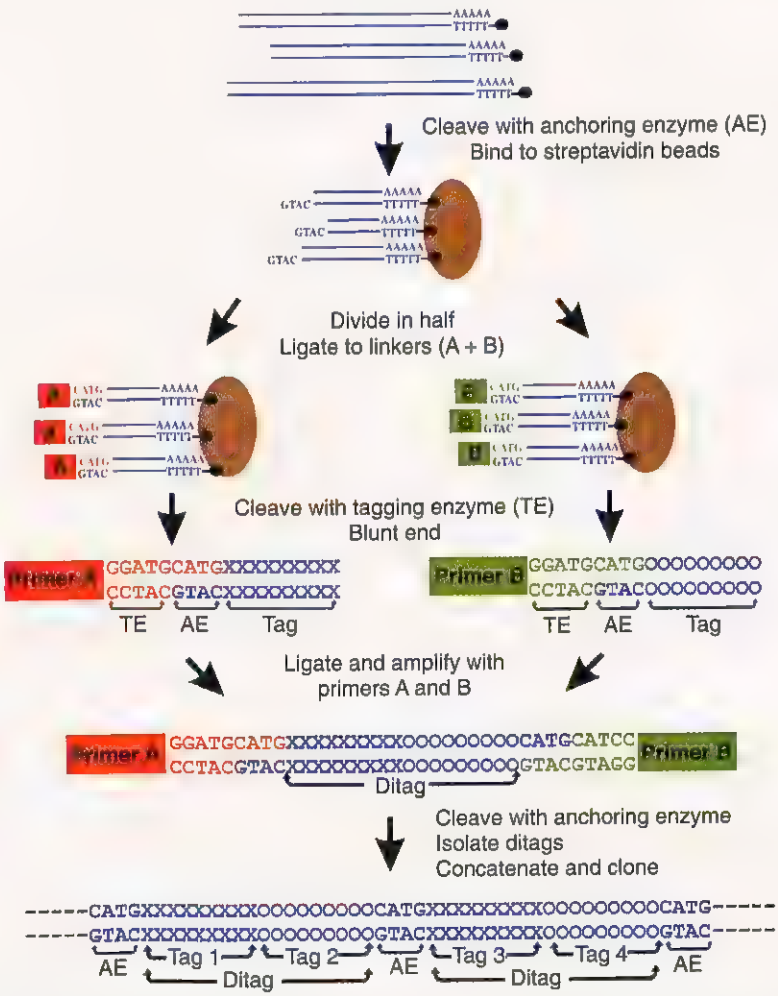

Fig. 1. Schematic of SAGE. The anchoring enzyme is Nla III and the tagging enzyme is Fok I. Sequences colored red and green represent primer-derived sequences, whereas blue represents transcript-derived sequences, with X and O indicating nucleotides of different tags. See text for further explanation.

V. E. Velculescu and K. W. Kinzler, Oncology Center and the Program in Human Genetics and Molecular Biology, Johns Hopkins University, Baltimore, MD 21231, USA. L. Zhang and B. Vogelstein, Howard Hughes Medical Institute, Johns Hopkins Oncology Center, Baltimore, MD 21231, USA.

*To whom correspondence should be addressed.

For example, Fig. 1 shows a combination of anchoring enzyme and tagging enzyme that would yield a 9-bp tag. After blunt ends were created, the two pools of released tags were ligated to each other. Ligated tags then served as templates for polymerase chain reaction (PCR) amplification with primers specific to each linker. This step served several purposes in addition to allowing amplification of the tag sequences. First, it provided for orientation and punctuation of the tag sequence in a very compact manner. The resulting amplification products contained two tags (one ditag) linked tail to tail, flanked by sites for the anchoring enzyme. In the final sequencing template, this resulted in 4 bp of punctuation per ditag. Second and most importantly, the analysis of ditags, formed before any amplification steps, provided a means to completely eliminate potential distortions introduced by PCR. Because the probability of any two tags being coupled in the same ditag is small, even for abundant transcripts, repeated ditags potentially produced by biased PCR could be excluded from analysis without substantially altering the final results. Cleavage of the PCR product with the anchoring enzyme allowed isolation of ditags that could then be concatenated by ligation, cloned, and sequenced.

As a demonstration of this approach, SAGE was used to characterize gene expression in the human pancreas. We chose Nla III as the anchoring enzyme and Bsm FI as the tagging enzyme, yielding a 9-bp tag (6). Computer analysis of human transcripts from GenBank indicated that greater than 95% of tags of this length were likely to be unique and that inclusion of two additional bases provided little additional resolution (7). As outlined above, mRNA from human pancreas was used to generate ditags (8) that were cloned into a plasmid vector (9). Clones containing at least 10 tags (range 10 to >50) were identified by PCR amplification and manually sequenced (10). Table 1 shows the analysis of the first 1000 tags. Sixteen percent were eliminated because they either had sequence ambiguities or were derived from linker sequences. The remaining 840 tags included 351 tags that occurred once and 77 tags that were identified multiple times (Table 1). Nine of the 10 most abundant tags matched at least one entry in GenBank release 87 (Table 1). The remaining tag was subsequently shown to be derived from amylase (see below). All 10 transcripts were derived from genes of known pancreatic function, and their prevalence was consistent with previous analyses of pancreatic RNA through conventional approaches (11).

The quantitative nature of SAGE was evaluated by construction of an oligo(dT)-primed pancreatic cDNA library that was screened with cDNA probes for trypsinogen 1 and 2, procarboxypeptidase A1, chymotrypsinogen, and elastase IIIB and protease

Table 1. Pancreatic SAGE tags. Tag indicates the 9-bp sequence identifying each tag, adjacent to the 4-bp anchoring Nla III site. *n* and Percent indicate the number of times the tag was identified and its frequency, respectively. Gene indicates the description and accession number of the GenBank release 87 entry found to exactly match the indicated tag when the SAGE software group was used, with the following exceptions. When multiple entries were identified because of duplicated entries (7), only one entry is listed. For chymotrypsinogen and trypsinogen 1, other genes (adenosine triphosphatase and myosin alkali light chain, respectively) were identified that were predicted to contain the same tags, but subsequent hybridization and sequence analysis identified the listed genes as the source of the tags. Alu entry indicates a match with a GenBank entry for a transcript that contained at least one copy of the Alu consensus sequence (15).

Tag	Gene	*n*	Percent
GAGCACACC	Procarboxypeptidase A1 (X67318)	64	7.6
TTCTGTGTG	Pancreatic trypsinogen 2 (M27602)	46	5.5
GAACACAAA	Chymotrypsinogen (M24400)	37	4.4
TCAGGGTGA	Pancreatic trypsin 1 (M22612)	31	3.7
GCGTGACCA	Elastase IIIB (M18692)	20	2.4
GTGTGTGCT	Protease E (D00306)	16	1.9
TCATTGGCC	Pancreatic lipase (M93285)	16	1.9
CCAGAGAGT	Procarboxypeptidase B (M81057)	14	1.7
TCCTCAAAA	No match (see Table, 2, P1)	14	1.7
AGCCTTGGT	Bile salt stimulated lipase (X54457)	12	1.4
GTGTGCGCT	No match	11	1.3
TGCGAGACC	No match (see Table 2 P2)	9	1.1
GTGAAACCC	21 Alu entries	8	1.0
GGTGACTCT	No match	8	1.0
AAGGTAACA	Secretory trypsin inhibitor (M11949)	6	0.7
TCCCCTGTG	No match	5	0.6
GTGACCACG	No match	5	0.6
CCTGTAATC	M91159, M29366, 11 Alu entries	5	0.6
CACGTTGGA	No match	5	0.6
AGCCCTACA	No match	5	0.6
AGCACCTCC	Elongation factor 2 (Z11692)	5	0.6
ACGCAGGGA	No match (see Table 2, P3)	5	0.6
AATTGAAGA	No match (see Table 2, P4)	5	0.6
TTCTGTGGG	No match	4	0.5
TTCATACAC	No match	4	0.5
GTGGCAGGC	NF-κB (X61499), Alu entry (S94541)	4	0.5
GTAAAACCC	TNF receptor II (M55994), Alu entry (X01448)	4	0.5
GAACACACA	No match	4	0.5
CCTGGGAAG	Pancreatic mucin (J05582)	4	0.5
CCCATCGTC	Mitochondrial CytC oxidase (X15759)	4	0.5
SAGE tags occurring:	Greater than three times	380	45.2
	Three times (15 × 3 =)	45	5.4
	Two times (32 × 2 =)	64	7.6
	One time	351	41.8
	Total SAGE tags	840	100.0

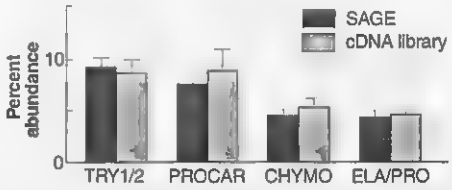

Fig. 2. Comparison of transcript abundance. Bars represent the percent abundance as determined by SAGE (dark bars) or hybridization analysis (light bars). SAGE quantitations were derived from Table 1 as follows: TRY1/2 includes the tags for trypsinogen 1 and 2; PROCAR indicates tags for procarboxypeptidase A1; CHYMO indicates tags for chymotrypsinogen; and ELA/PRO includes the tags for elastase IIIB and protease E. The cDNA hybridizations were as described (12). Error bars represent the standard deviation determined by taking the square root of counted events and converting it to a percent abundance. A Poisson distribution was assumed.

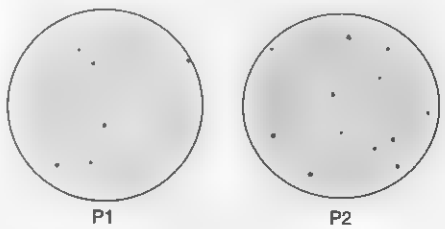

Fig. 3. Screening a cDNA library with SAGE tags. P1 and P2 show typical hybridization results obtained with 13-bp oligonucleotides as described (13). P1 and P2 correspond to the transcripts described in Table 2. Images were obtained with a Molecular Dynamics PhosphorImager, and the circle indicates the outline of the filter membrane to which the recombinant phage were transferred before hybridization.

E (*12*). The relative abundance of the SAGE tags for these transcripts was in good agreement with the results obtained with library screening (Fig. 2). Furthermore, whereas neither trypsinogen 1 and 2 nor elastase IIIB and protease E could be distinguished by the cDNA probes used to screen the library (*12*), all four transcripts could readily be distinguished on the basis of their SAGE tags (Table 1).

In addition to providing quantitative information on the abundance of known transcripts, SAGE could be used to identify novel expressed genes. Although for the purposes of SAGE only the 9-bp sequence identifying each transcript was considered, each SAGE tag defines a 13-bp sequence composed of the anchoring enzyme (4-bp) site plus the 9-bp tag. As an illustration of this potential, 13-bp oligonucleotides were used to isolate the transcripts corresponding to four unassigned tags (P1 to P4), that is, tags without corresponding entries from GenBank release 87 (Table 1). In each of the four cases, it was possible to isolate multiple cDNA clones for the tag by simply screening the pancreatic cDNA library with the 13-bp oligonucleotide as hybridization probe (examples in Fig. 3) (*13*). In each case, sequencing of the derived clones identified the correct SAGE tag at the predicted 3′ end of the identified transcript. The abundance of plaques identified by hybridization with the 13-bp oligonucleotides was in good agreement with that predicted by SAGE (Table 2). Tags P1 and P2 were shown to correspond to amylase and preprocarboxypeptidase A2, respectively. No entry for preprocarboxypeptidase A2 and only a truncated entry for amylase was present in GenBank release 87, thus accounting for their unassigned characterization. Tag P3 did not match any genes of known function in GenBank but did match numerous ESTs, providing further evidence that it represented a real transcript. The cDNA identified by P4 showed no significant similarities, suggesting that it represented a previously uncharacterized pancreatic transcript.

These results demonstrate that SAGE can provide both quantitative and qualitative data about gene expression. The combination of different anchoring enzymes with various recognition sites and type IIS enzymes with cleavage sites 5 to 20 bp from their recognition elements lends great flexibility to this strategy. As efforts to fully characterize the genome near completion, SAGE should allow a direct readout of expression in any given cell type or tissue. In the interim, we envision that the major application of SAGE will be the comparison of gene expression patterns in various developmental and disease states. Any laboratory with the capability to perform PCR and manual sequencing could perform SAGE for this purpose. Adaptation of this technique to an automated sequencer would allow the analysis of over 1000 transcripts in a single 3-hour run (*14*).

The appropriate number of tags to be determined will depend on the application. For example, the definition of genes expressed at relatively high levels (0.5% or more) in one tissue, but low in another, would require only a single day. Determination of transcripts expressed at greater than 100 mRNAs per cell (0.025%) should be quantifiable within a few months by a single investigator. Use of different anchoring enzymes will ensure that virtually all transcripts of the desired abundance can be identified. The genes encoding those tags shown to be most interesting on the basis of their differential representation can be positively identified by a combination of database searching, hybridization, and sequence analysis as demonstrated in Tables 1 and 2. Obviously, SAGE could also be applied to the analysis of organisms other than humans and could direct investigation toward genes expressed in specific biologic states.

Table 2. Characterizations of unassigned SAGE tags. Tag and SAGE Abundance are as described in Table 1; 13-mer hyb. indicates the results obtained by screening a cDNA library with a 13-bp oligonucleotide (*13*). The number of positive plaques divided by the total plaques screened is indicated in parenthesis after the percent abundance. A positive in the SAGE Tag column indicates that the expected SAGE tag was identified at the 3′ end of isolated clones. Description indicates the results of BLAST searches of the daily updated GenBank entries at NCBI (National Center for Biotechnology Information) as of 9 June 1995 (*16*). A description and accession number are given for the most significant matches. P1 was found to match a truncated entry for amylase, and P2 was found to match an unpublished entry for preprocarboxypeptidase A2 that was entered after GenBank release 87.

Tag	Abundance (%)		SAGE tag	Description
	SAGE	13-mer hyb.		
P1 TCCTCAAAA	1.7	1.5 (6/388)	+	3′ end of pancreatic amylase (M28443)
P2 TGCGAGACC	1.1	1.2 (43/3700)	+	3′ end of preprocarboxypeptidase A2 (U19977)
P3 ACGCAGGGA	0.6	0.2 (5/2772)	+	EST match (R45808)
P4 AATTGAAGA	0.6	0.4 (6/1587)	+	No match

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6. Bsm FI was predicted to cleave the complementary strand 14 bp 3′ to the recognition site GGGAC and to yield a 4-bp 5′ overhang (New England BioLabs). Overlapping the Bsm FI and Nla III (CATG) sites as indicated (GGGACATG) would be predicted to result in an 11-bp tag. However, our analysis suggested that under our cleavage conditions (37°C rather than 65°C), Bsm FI often cleaved closer to its recognition site, leaving a minimum of 12 bp 3′ of its recognition site. Therefore, only the 9 bp closest to the anchoring enzyme site was used for analysis of tags.
7. Human sequences (84,300) were extracted from the GenBank release 87 database by means of the FINDSEQ program provided on the IntelliGenetics Bionet on-line service. All further analysis was performed with a SAGE program group written in Microsoft Visual Basic for the Microsoft Windows operating system. The SAGE database analysis program was set to include only sequences noted as "RNA" in the locus description and to exclude entries noted as "EST," resulting in a reduction to 13,241 sequences. Analysis of this subset of sequences with Nla III as anchoring enzyme indicated that 4127 of the 9-bp tags occurred only once, whereas 1511 tags were found in more than one entry. Nucleotide comparison of an arbitrarily chosen subset (100) of the latter entries indicated that at least 83% were a result of redundant database entries for the same gene or highly related genes (>95% identity over at least 250 bp). This suggested that 5381 of the 9-bp tags (95.5%) were particular to a transcript or highly conserved transcript family. Likewise, analysis of the same subset of GenBank with an 11-bp tag resulted in only a 6% decrease in repeated tags (1511 to 1425) instead of the 94% decrease expected if the repeated tags were due to unrelated transcripts.
8. Total pancreatic mRNA (5 μg, Clontech) was converted to double-stranded cDNA with a BRL cDNA synthesis kit. The manufacturer's protocol was used, except for the inclusion of primer biotin-5′-T_{18}-3′. The cDNA was then cleaved with Nla III and the 3′ restriction fragments isolated by binding to magnetic streptavidin beads (Dynal). The bound DNA was divided into two pools, and one of the following linkers was ligated to each pool: linker A, 5′-TTTTACCAGCTTATTCAATTCGGTCCTCTCGCACAGGGACATG-3′, 3′-dideoxyATGGTCGAATAAGTTAAGCCAGGAGAGCGTGTCCCT-5′; linker B, 5′-TTTTTGTAGACATTCTAGTATCTCGTCAAGTCGGAAGGGACATG-3′, 3′-dideoxyAACATCTGTAAGATCATAGAGCAGTTCAGCCTTCCCT-5′. After extensive washing to remove unligated linkers, the linkers and adjacent tags were released by cleavage with Bsm FI. The resulting overhangs were filled in with T4 polymerase, and the pools were combined and ligated to each other. The desired ligation product was then amplified for 25 cycles with 5′-CCAGCTTATTCAATTCGGTCC-3′ and 5′-GTAGACATTCTAGTATCTCGT-3′ as primers. The PCR reaction was then analyzed by polyacrylamide gel electrophoresis (PAGE) and the desired product excised. An additional 15 cycles of PCR were then performed to generate sufficient product for efficient ligation and cloning.
9. The PCR product was cleaved with Nla III and the band containing the ditags was excised and self-ligated. After ligation, the concatenated ditags were separated by PAGE and products greater than 200 bp were excised. These products were

cloned into the Sph I site of pSL301 (Invitrogen). Colonies were screened for inserts by PCR with T7 and T3 sequences located outside the cloning site as primers.

10. Selected clones were manually sequenced as described [G. Del Sal, G. Manfioletti, C. Schneider, *Biotechniques* **7**, 514 (1989)] with 5′-GACGTCGAC-CTGAGGTAATTATAACC-3′ as primer. Sequence files were analyzed by means of the SAGE software group (*7*), which identifies the anchoring enzyme site with the proper spacing and extracts the two intervening tags and records them in a database. The 1000 tags were derived from 413 unique ditags and 87 repeated ditags. The latter were counted only once to eliminate potential PCR bias of the quantitation, as described in the text.
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12. Pancreatic mRNA from the same preparation was used for SAGE and to construct a cDNA library in the ZAP Express vector. The ZAP Express cDNA Synthesis kit (Stratagene) was used according to the manufacturer's protocol. Analysis of 15 randomly selected clones indicated that 100% contained cDNA inserts. Plates containing 250 to 500 plaques were hybridized as described [J. M. Ruppert *et al.*, *Mol. Cell. Biol.* **8**, 3104 (1988)]. The cDNA probes for trypsinogen 1, trypsinogen 2, procarboxypeptidase A1, chymotrypsinogen, and elastase IIIB were derived by RT-PCR from pancreas RNA. The sequences of primers are available from the authors upon request. The trypsinogen 1 and 2 probes were 93% identical and hybridized to the same plaques under the conditions used. Likewise, the elastase IIIB probe was >95% identical to protease E.
13. Plates containing 250 to 2000 plaques were hybridized to oligonucleotide probes with the same conditions previously described for standard probes except that the hybridization temperature was reduced to room temperature (*12*). Washes were performed in 6× standard saline citrate–0.1% SDS for 30 min at room temperature. The probes consisted of 13-bp oligonucleotides that were labeled with [γ^{32}-P]ATP through use of T4 polynucleotide kinase
14. An ABI 377 sequencer can produce a 451-bp read for 36 templates in a 3-hour run [(451 bp/11 bp per tag) × 36 = 1476 tags].
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17. Supported by NIH grants CA57345, CA35494, GM07309. B.V. is an American Cancer Society Research Professor and an Investigator of the Howard Hughes Medical Institute. We thank S. Kern, B. D. Nelkin, and members of our laboratories for their critical review and valuable discussions

14 June 1995; accepted 11 August 1995

TECHNICAL COMMENT

The Radius of Gyration of an Apomyoglobin Folding Intermediate

Apomyoglobin (apoMb) forms a stable compact partially folded state under acidic conditions (*1*). This "molten globule" intermediate is slightly expanded relative to the native form of the protein, with a radius of gyration (R_g) of 23 (± 2) Å versus 19 (± 1) Å (*2*), and shows stable secondary structure (*3*) in the A, G, and H helices (Fig. 1).

We demonstrated recently, with the use of stopped-flow circular dichroism and pulse-labeling hydrogen exchange measurements, that the earliest detectable intermediate (formed within 6 ms) in the apoMb kinetic refolding pathway closely resembles the equilibrium molten globule state populated under acid conditions (*4*). A key question remained as to how compact this kinetic intermediate is compared to the equilibrium and native states. The cooperative unfolding of the kinetic intermediate and the significant protection from amide proton exchange (as compared to corresponding isolated peptides in solution) led us to propose that the kinetic intermediate is also compact (*4*, *5*). Such a proposal could best be verified by direct determination of the size of the protein as it folds, but measurements of this nature were not feasible at the time.

Newly developed improvements in time-resolved small angle x-ray scattering (SAXS) experiments allow direct measurement of the time-dependent change of R_g of a protein as it folds in the millisecond to second time frame (*6*, *7*). We initiated studies of the refolding of apoMb using this technique, under conditions similar to those employed in our previous work (*4*). SAXS data collected during the first 100 ms after initiation of the refolding reaction (*8*) are shown in Fig. 2.

Data collected from the fully refolded protein and unfolded protein are given for comparison (Fig. 2). The data obtained 100 ms after the initiation of folding are within experimental error of the data obtained for the refolded protein, and easily distinguishable from data obtained for the unfolded state. An R_g value of 23 (± 2) Å is obtained at 100 ms, only 1 Å greater than the 22 (± 1) Å value obtained for the refolded protein. By contrast, the unfolded state has an R_g of 34 (± 2) Å. The slightly higher than expected R_g value obtained for the refolded state may result from either experimental error (*9*) or a small degree of sample aggregation owing to radiation damage during exposure. It is possible that the R_g value obtained at 100 ms is similarly inflated, and it may therefore be considered an upper bound on the true R_g.

Our conclusion that the intermediate is compact is based on the small differences

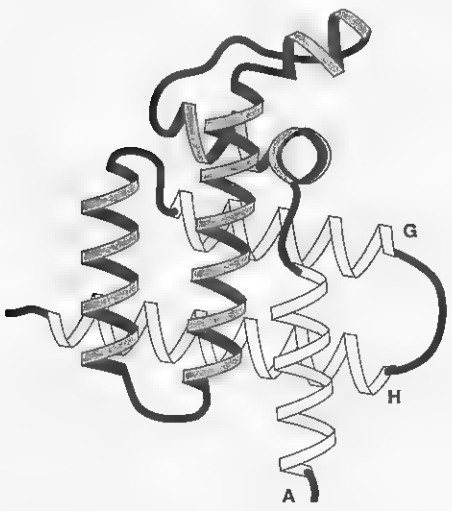

Fig. 1. Sketch of the structure of holo-myoglobin, illustrating the location of the A, G, and H helices, which are present in both the equilibrium and kinetic folding intermediates of the apoprotein.

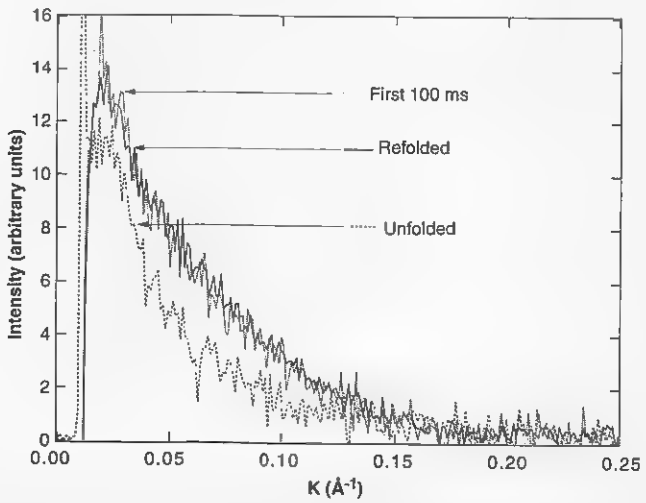

Fig. 2. SAXS data from sperm whale apomyoglobin after 100 ms of folding, after 4.2 s of folding, and in the unfolded state. Detected intensity is plotted as a function of *K*. Data from the unfolded state is scaled to match the folded state data at zero scattering angle. The data obtained from the fully folded protein and that obtained after 100 ms of folding are barely distinguishable from each other and are different from the data for the unfolded protein.

between both the raw SAXS data and the R_g values from the kinetic intermediate and from the fully refolded protein. It is unnecessary to invoke specific models to reach this conclusion. Indeed, the low resolution of SAXS data and the uncertainties inherent in time-resolved SAXS measurements make it both inappropriate and unwise to attempt to interpret the current data in terms of specific structural models.

Taken as an upper bound, the 23 (± 2) Å R_g obtained at 100 ms illustrates that the first intermediate observed in the kinetic refolding reaction of apoMb is at least as compact as the equilibrium "molten globule" state of apoMb. The fact that this R_g value is only 1 Å greater than the value measured for the fully refolded protein, together with the great similarity of the actual SAXS data at 100 ms to the data from the refolded protein, suggests that the kinetic intermediate may be nearly as compact as the native state itself (*10*). SAXS data collected during the first 20 ms of folding indicate these same results, but with a lower signal to noise ratio (*11*). Thus the present experiments provide a direct measurement of the size of the early kinetic folding intermediate of apoMb.

David Eliezer
*Patricia A. Jennings**
Peter E. Wright
Department of Molecular Biology, The Scripps Research Institute, La Jolla, CA 92037, USA

Sebastian Doniach
Department of Applied Physics, Stanford University, Stanford, CA 94305, USA
Keith O. Hodgson
Department of Chemistry and Stanford Synchrotron Radiation Laboratory, Stanford University
Hirotsugu Tsuruta
Stanford Synchrotron Radiation Laboratory

*Present address: Department of Chemistry and Biochemistry, University of California, San Diego, La Jolla, CA 92037, USA

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7. Data were collected at the Stanford Synchrotron Radiation Laboratory with the use of a new high-flux multi-layer x-ray monochromator calibrated to a photon energy of 8980 electron volts. The bandwidth of radiation transmitted by this monochromator is 10-fold greater than standard Si(III) double-crystal monochromators, providing the additional flux necessary for studies at protein concentrations low enough to avoid dimerization of highly association-prone folding intermediates.
8. Refolding was triggered by rapid dilution of 10 mg/ml protein in 5.6 M urea to 1.4 mg/ml protein in 0.8 M urea. The dead time of the rapid mixer (Unisoku Inc., Osaka) is on the order of 10 ms. Kinetic data were accumulated from 1200 individual mixing events. Radii of gyration were extracted from the background-subtracted data using Guinier fits to the region $K = 0.034$ to $K = 0.063$ where K, the scattering vector amplitude, equals $4\pi\sin(\theta/2)/\lambda$ (θ is the scattering angle and λ is the x-ray photon wavelength).
9. The lowest possible protein concentration was used to prevent the possible oligomerization of kinetic folding intermediates. An unfortunate consequence of such a low concentration is that a much larger fraction of the detected x-ray photons are from background sources, leading to larger experimental errors.
10. In such a highly compact intermediate it seems likely that the polypeptide chain segment that forms helices B through F in native myoglobin would also have undergone some degree of collapse. The possibility that the kinetic intermediate may be more compact than the equilibrium intermediate is supported by our previous observation (*5*) that the apoMb equilibrium intermediate would be about 1 kcal/mol more stable under the conditions used for the kinetic studies than under the partially denaturing conditions in which it is typically studied.
11. The time-resolved circular dichroism and amide exchange data (*4*) indicate a lag phase between 5 and 350 ms into the folding reaction, where no change in mean residue ellipticity or amide proton protection is observed
12. SSRL is supported by the U.S. Department of Energy, Office of Basic Energy Sciences, and Office of Health and Environmental Research, and by the NIH Biomedical Research Technology Program. Additional funding was provided by the National Institutes of Health through grant numbers DK34909 (to P.E.W.) and RR01209 (to K.O.H.) and through postdoctoral award GM14541 (to P.A.J.).

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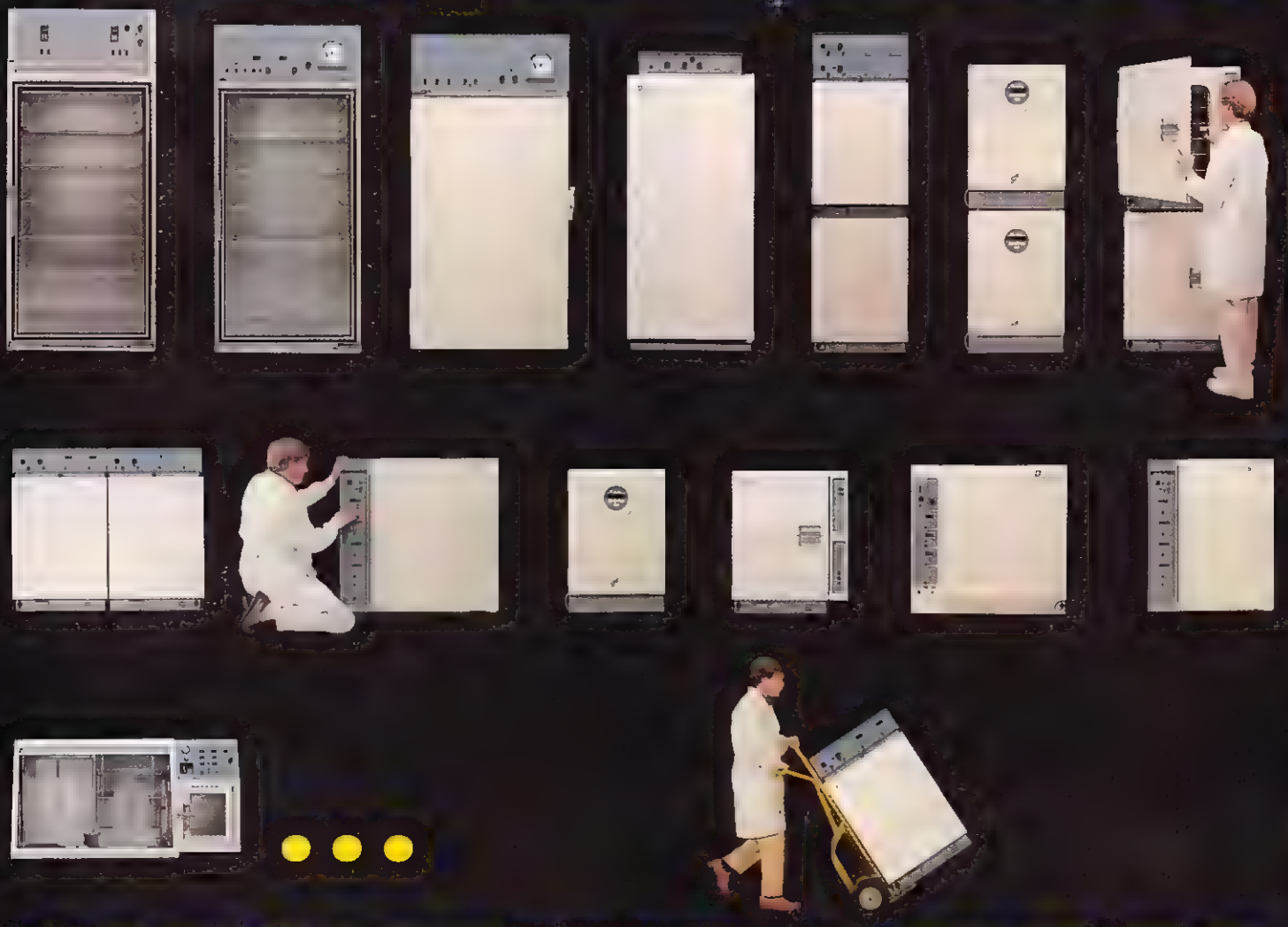

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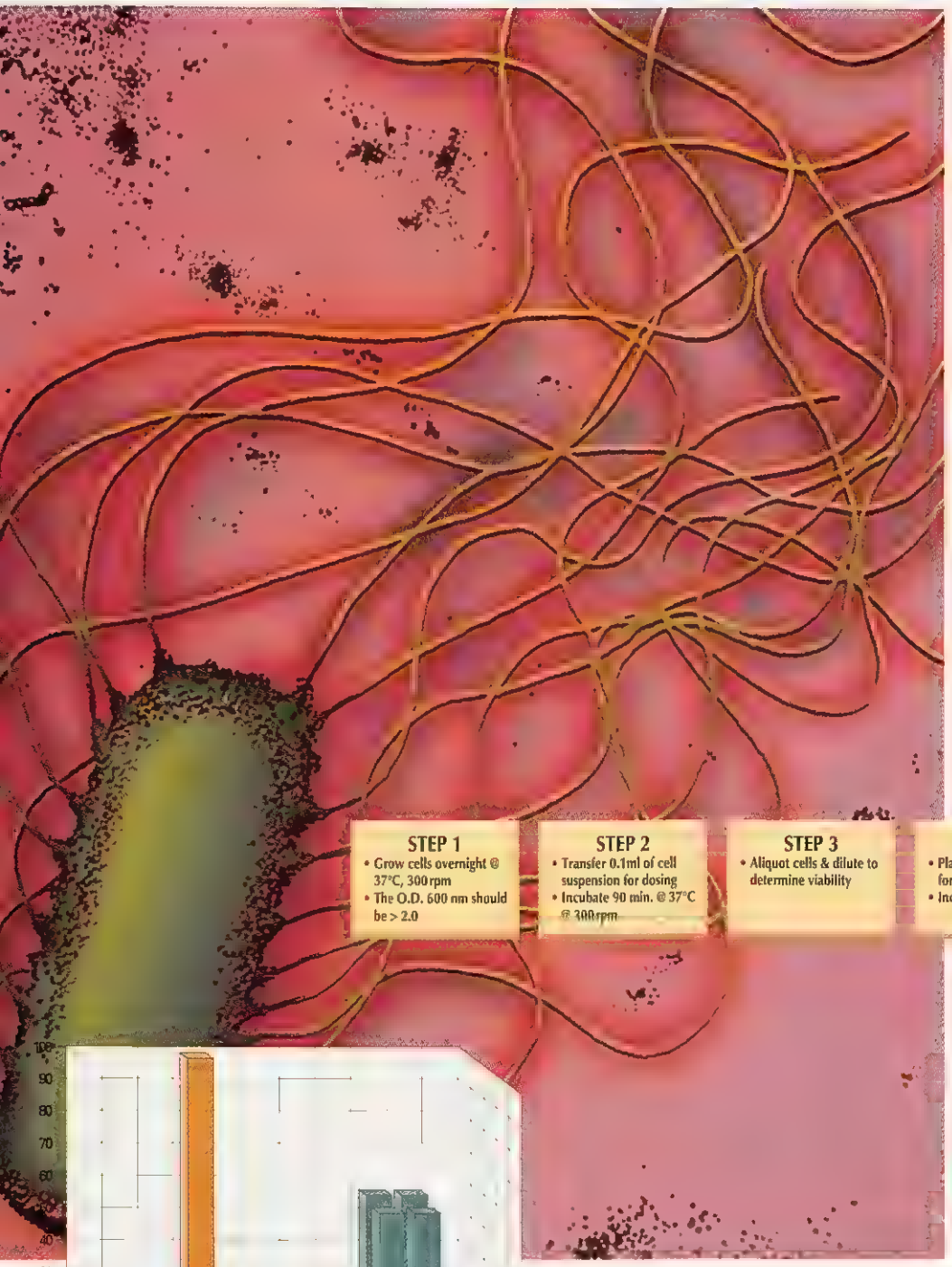

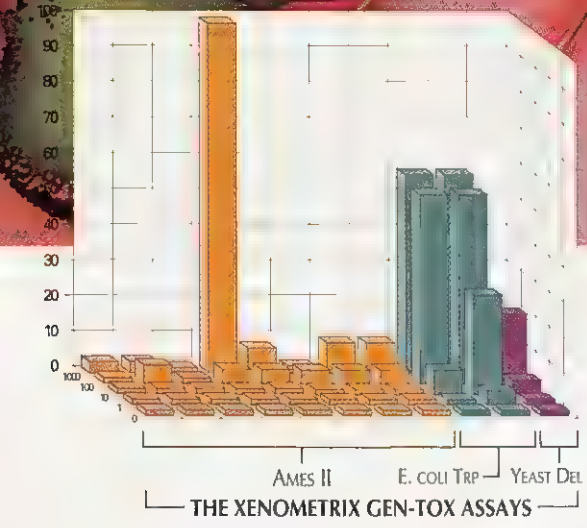

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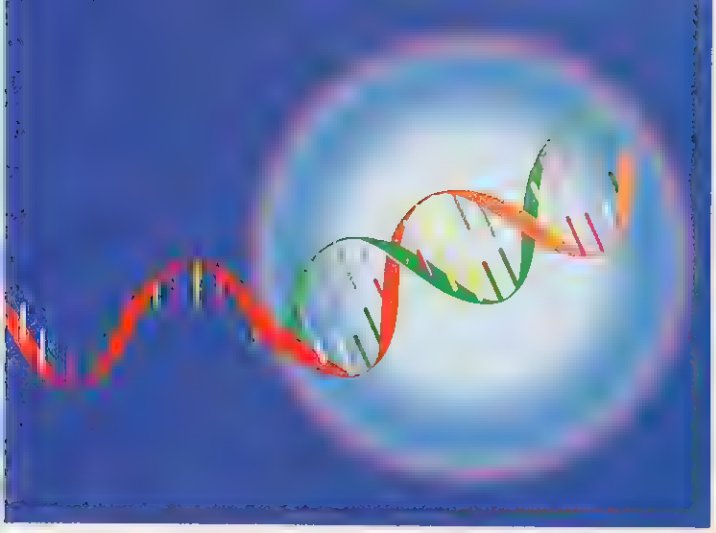

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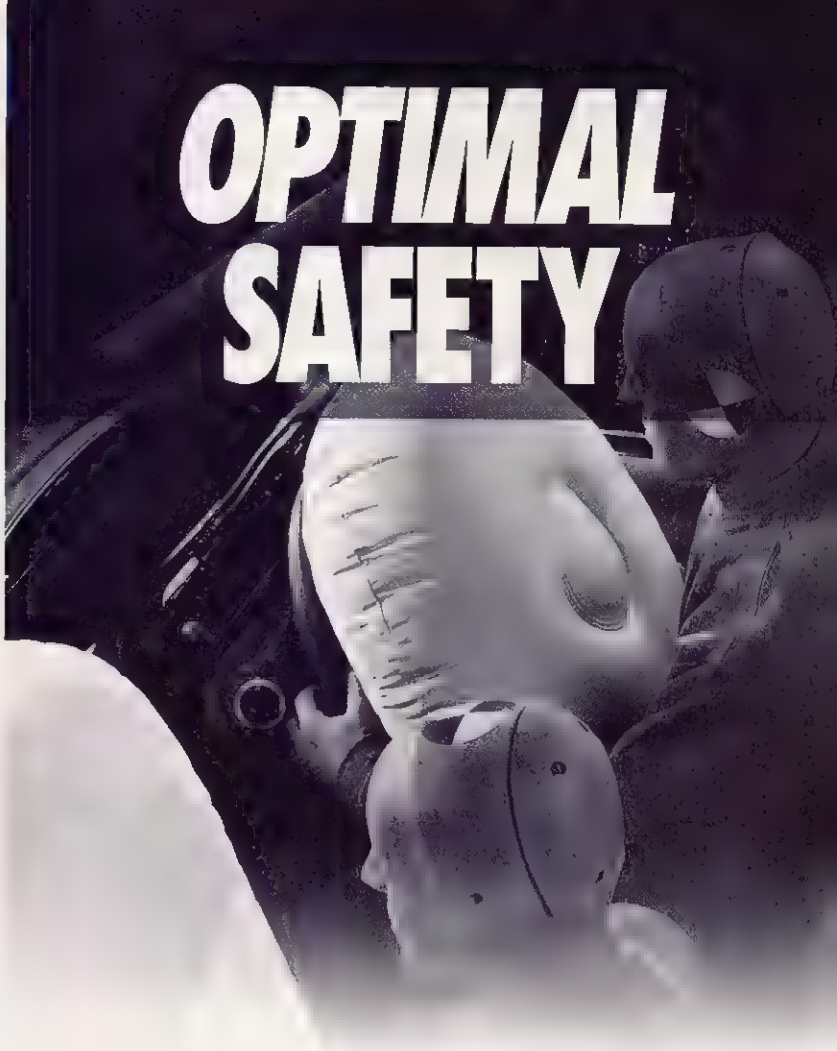

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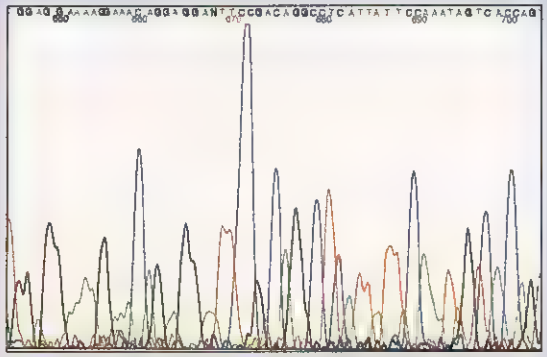

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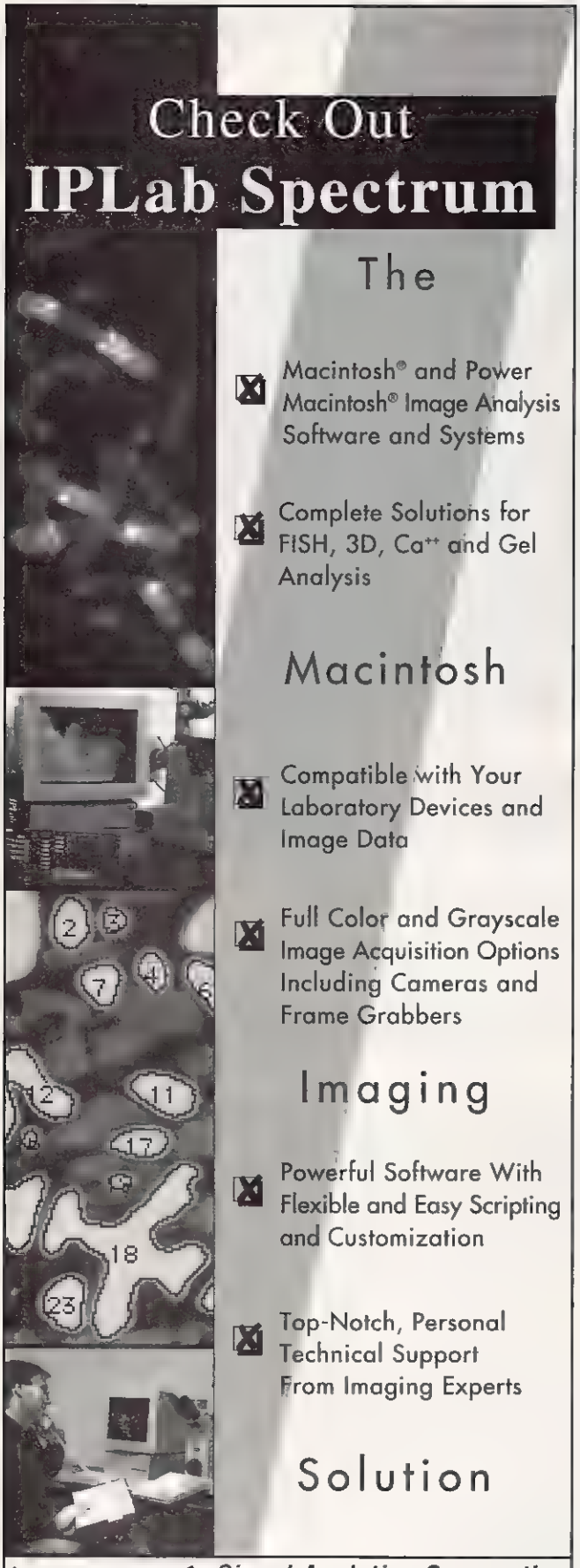

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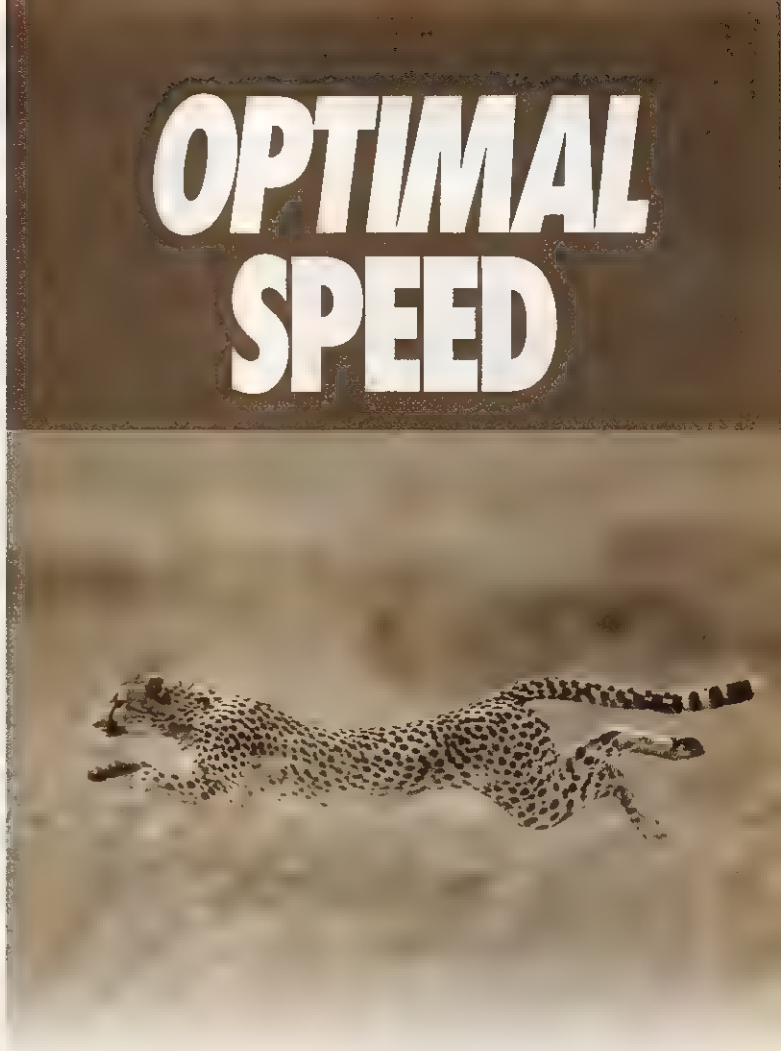

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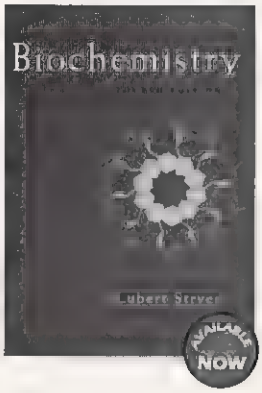

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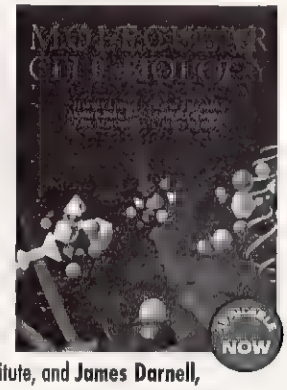

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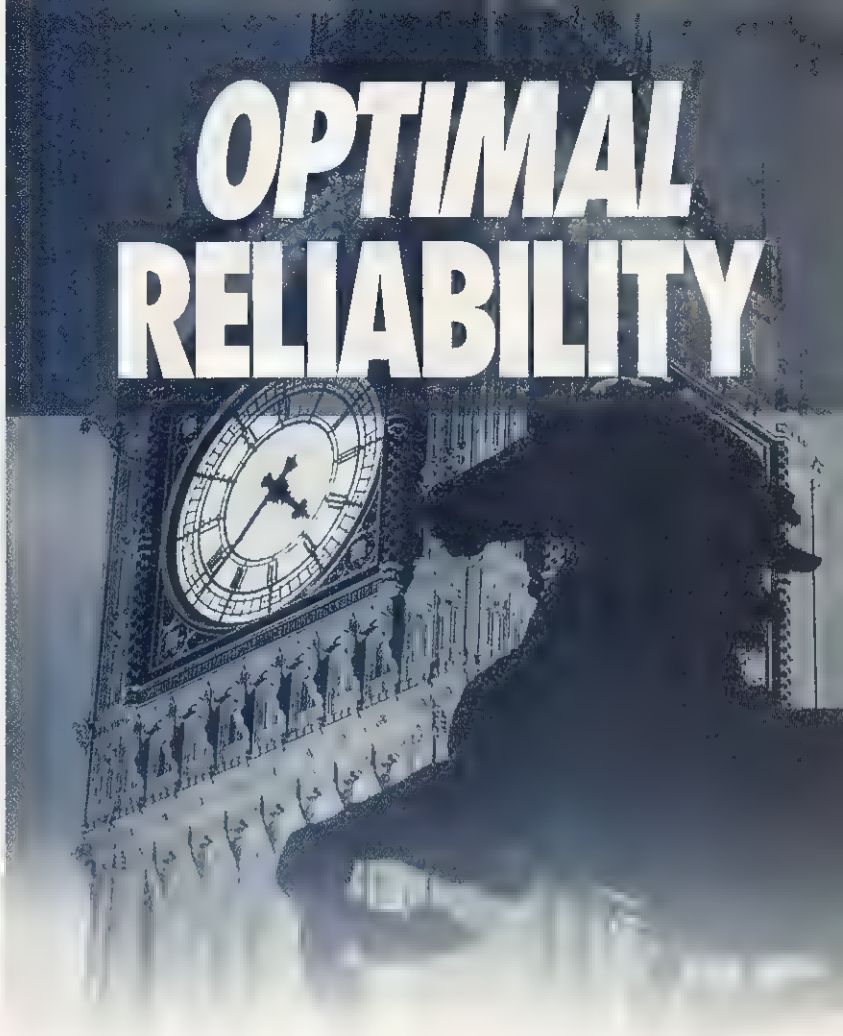

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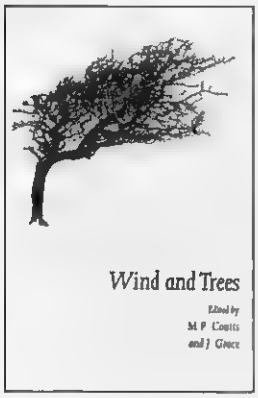

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BOOK REVIEWS

The NORC Sex Survey

The Social Organization of Sexuality. Sexual Practices in the United States. EDWARD O. LAUMANN, JOHN H. GAGNON, ROBERT T. MICHAEL, and STUART MICHAELS. University of Chicago Press, Chicago, 1994. xxxii, 718 pp., illus. $49.95 or £39.95.

Sex in America. A Definitive Survey. ROBERT T. MICHAEL, JOHN H. GAGNON, EDWARD O. LAUMANN, and GINA KOLATA. Little Brown, New York, 1994. x, 300 pp., illus. $22.95; paperback edition, Warner, New York, 1995, $12.99.

The publication of these two volumes in 1994 attracted a great deal of attention. Cover stories in *Time* and *U.S. News & World Report* and front-page articles in many newspapers summarized the data reported in the books. The results of the National Health and Social Life Survey (NHSLS) are quickly being incorporated into our conventional wisdom about sexuality in America. But before we accept them as "the truth about Americans and sex" (*Time*, 17 Oct. 1994) we need to ask how well the survey stands up as science.

According to the dust jacket of *The Social Organization of Sexuality*, the NHSLS is "the nation's most comprehensive, representative survey of sexual behavior in the general adult population of the United States." It is comprehensive; the 90-minute interview included questions about a wide variety of sexual attitudes and behaviors. It is also representative; it is based on a stratified, multistage area probability sample of clusters of households, the state-of-the-art technique in survey research. Within the selected households, one English-speaking adult between 18 and 59 years of age was randomly selected as the respondent. Interviews were completed with 3342 persons, 78.6% of those eligible to participate.

The research was carefully designed. The questionnaire, reproduced in an appendix in both books, was written in "standard English," rather than slang or technical language. Terms were defined for the respondents the first time they were used. To minimize problems of recall, highly detailed questions were asked about sexual partners and behavior only for the 12 months immediately preceding the interview. The interviewing was conducted by the National Opinion Research Center (NORC), a widely respected scientific survey organization. The interviewers were primarily persons who had worked for NORC previously, and each received at least three days of training in the specifics of the NHSLS.

The questionnaire content and the data analyses reported in both books were guided by a social-constructionist theoretical orientation. Three specific theories are used consistently in the interpretation of results. Sexual script theory (developed by Gagnon) suggests that culturally based scripts influence what kinds of people we select as partners and what behaviors we engage in. Choice theory (Michael) proposes that sexual behavior reflects individual goals and opportunities. Social network theory (Laumann) is used to explain what types of people do and do not develop sexual relationships with one another. These are presented as alternatives to the biological perspective that the authors claim (incorrectly) has dominated prior research on sexual behavior.

The authors acknowledge that scripts, rational decision-making, and composition of social networks cannot be measured in a population survey. Instead, they use the demographic characteristics of gender, age, marital status, education, religious preference, and race/ethnicity as "master statuses."

> Our approach is to focus on differences across the "status groups" defined by the master statuses in sexual behaviors, attitudes, and partnering activity in an attempt to infer the existence of different scripts, choices, and network structures [Laumann *et al.*, p. 31].

The Social Organization of Sexuality was written for a scientific audience. It begins by describing the theories and the design of the research. The remaining 12 chapters present results: sexual practices (behavior), number of partners, sexual networks, homosexuality, formative experiences including coerced sex, sexual health (satisfaction, dysfunctions), sexually transmitted infections, sexual unions (cohabitation, marriage), and sexual attitudes. In each chapter, tables and graphs present the distribution of responses on relevant measures according to the master statuses. There are usually substantial differences by gender, age, and marital status and often differences by race. These differences are consistently interpreted as reflecting the impact of sexual scripts, choices and opportunities, and network ties, especially ties to other "stakeholders," such as spouse/partner or parents. Thus, most couples are homophilous (that is, similar) in race, a finding that is said to reflect the strong pressures exerted by parents, friends, ministers, and others against interracial relationships. Frequently two or more of the master statuses are related to a measure such as frequency of sexual activity, leading the reader to ask what the contribution of each is, the other being controlled for. Too often, the analyses do not answer that question. Multivariate analyses, logistic regressions, are reported in only a few cases.

Highlights of the results: the modal respondent engaged in sexual activity "a few times a month"; vaginal intercourse appeals to more men and women than any other sexual practice; 80% of those interviewed had no or one sexual partner in the preceding year; 25% of the married men and 10% of the married women reported extramarital sexual activity; most sexual partnerships involve people who are of the same race and religion and similar in education and age; 4.9% of the men and 4.1% of the women report having had sexual activity with a same-gender partner since age 18; people are engaging in intercourse for the first time at younger ages; people with one sexual partner are happier than people with none or more than one; from 4.7% to 49% of various subgroups engage in sexual activity with partners who are relatively unknown to them; and 50% of cohabiting relationships last less than one year.

The presentation of the results is confusing at times. There is considerable commentary interspersed with the data. There are comparisons with the data reported by Kinsey in 1948 and 1953, discussions of the historical context of particular findings, analyses of the social or epidemiological significance of behaviors, and summaries of additional analyses. Though at times these make for interesting reading, they often make it difficult to grasp the principal results. The presentation also suffers from inconsistent use of italics to highlight major findings and from the lack of substantive concluding sections at the ends of several of the chapters.

Sex in America was written for nonscientific audiences. It presents the same general results, using a small number of illustrations and tables. It contains much less technical detail. The presentation consistently contrasts what are said to be popular myths about sex with the findings of the survey. The data are supplemented with real-life examples and vignettes, drawn from films, newspaper articles and columns, and books. The emphasis throughout is on how sexual behaviors and relationships are socially patterned. There is no obvious organization of the material within or across chapters.

Vignette: Song of Ourselves

If Walt Whitman were with me, I bet he'd sing the song of the sequences metapattern. He would see each detail of the prophase, metaphase, anaphase, and telophase sequence of a cell's arrow of mitosis. He would be there through all the explosive stages of a rocket. He would molt with the caterpillar through each instar of its growth and follow the path of aluminum from ore to foil. He would sing the stages of personal development in the theories of Piaget and Erickson, and the stages of cosmic creation in main-sequence stars. Embryo development from morula to blastula and gastrula; energy shuttled along the cytochrome chain in the membrane of a chloroplast; state formation, power consolidation, imperialization, and collapse in archeology; Carlos Castaneda's progression through the gates of dreaming; the sequential splitting of the four forces of physics in the early universe; the genetic leaps that altered the wild teosinte into maize; the primary, secondary, and tertiary steps that take proteins from amino acid chains into folded, globular forms ready for action; the computer bucket brigades of genetic algorithms; and for every human being the painful and glorious alchemical steps toward individuation—all these and more our friend Walt would extol.

—*Tyler Volk*, in *Metapatterns, across Space, Time, and Mind* (Columbia University Press)

In the year since its publication, *Social Organization* has generated several controversies. The most fundamental concerns the validity of the data, which are based entirely on what the respondents said in answer to the questions. Direct methods of verifying reports of sexual behavior do not exist. Laumann and colleagues used several indirect methods. First, they compared the answers of people who readily agreed to be interviewed with those of people who were "reluctant." Only 2 of 30 comparisons revealed statistically significant differences. Second, the researchers compared reports of sexual activity in the NHSLS to those obtained by a 1991 NORC survey; only one of 22 comparisons revealed statistically significant differences. Finally, the authors note numerous instances where their findings are similar to the results reported by other researchers. Some critics are unconvinced. Some design features may have reduced honesty, such as not matching interviewer and respondent on gender or race and allowing the interview to be conducted when others were physically present (in 21% of the cases). Limited evidence indicates that matching interviewer and respondent does not affect reported sexual behavior. The extreme view is that self-reports of sexual behavior will never be truthful and that surveys such as this one are of questionable value. I believe that the NHSLS used the best current survey technology and that the data have a high degree of internal validity. Exact numbers, such as the mean number of sexual partners since age 18, may not be accurate, but the ordering of respondents on the resulting scale probably is.

A second controversy concerns the conclusions that Laumann and colleagues reach about the threat of HIV infection. The findings that 80% of the respondents have no or one sexual partner per year and that most partnerships involve people of similar age, education, and race/ethnicity lead to the inference that there are few if any persons whose sexual contacts "bridge" the boundary between groups where rates of infection are high and groups where the rates are low. In *Social Organization*, the conclusion is stated as follows:

> We are suggesting ... that the general lack of connectivity present in sexual networks among adults in the United States, together with the relatively low transmission probability of AIDS through vaginal intercourse, will significantly restrict the extent to which this disease will spread into the general population [p. 282].

In *Sex in America*, the statement is less tentative:

> We believe ... that AIDS is, and is likely to remain, confined to exactly the risk groups where it began: gay men, intravenous drug users and their sexual partners. We are convinced that there is not and very unlikely ever will be a heterosexual AIDS epidemic in this country [p. 216].

Several observers, including public health experts, are critical of this conclusion. Even if the reasoning is correct, there are infected middle-class heterosexuals. The problem for any individual is uncertainty about the sexual history of his or her next partner. Heterosexuals are at risk, and it is a disservice to make statements that encourage them to be less vigilant. Furthermore, other aspects of the NHSLS data are inconsistent with this conclusion. The probability of HIV transmission is much greater through anal intercourse, and 10% of the heterosexual men and 9% of the heterosexual women report that behavior in the preceding 12 months. As noted earlier, up to 49% of various subgroups engage in sexual activity with unfamiliar partners. Furthermore, the NHSLS sample excludes the homeless and persons in institutions such as colleges, the military, and prisons. The rate of infection may be greater in these groups, and members may have sexual partners who are less similar in age, race/ethnicity, and education. Certainly if significant numbers of college students become infected there will be a heterosexual AIDS epidemic, because the pattern of multiple partners is widespread among college students.

A third controversy is over the incidence of homosexuality in the population. For two decades, the commonly accepted figure has been that 10% of the population is homosexual. In recent years, scholars have argued for a multidimensional definition of sexual orientation, one that includes self-identification, the gender of sexual partners, and preference. The NHSLS uses this approach; thus the answer to the question "how many" is "it depends." In the survey, 2.8% of the males and 1.4% of the females identify themselves as homosexual, 4.9% and 4.1% report having had a same-gender partner since age 18, and 4.4% and 5.6% report that same-gender sex is "very appealing." The largest percentage is only about half of the commonly accepted 10%. Some critics have cited this as evidence that respondents were not truthful, arguing that these percentages should be much larger. However, the NHSLS results are very similar to those reported by several other studies of American samples and by the recent British and French surveys of sexual activity. Laumann and colleagues present data showing that the incidence of male-male sexual activity since age 18 is 16.4% in the 12 largest central cities; residents of those cities will correctly perceive that more than 5% of their male population is gay.

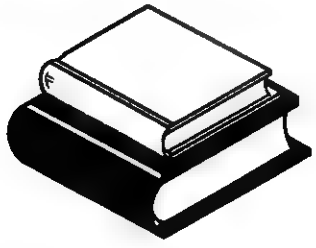
The fourth controversy is over the contribution of the NHSLS relative to other research on sexual behavior in the past 30 years. Laumann and colleagues make sweeping claims in this regard (for example, *Sex in America* is subtitled "The Definitive Survey"). They dismiss most prior research as not comprehensive and representative. However, there are at least ten prior surveys that have utilized probability sampling techniques and several that have included questions about a broad range of sexual

activity. These studies have made important contributions; in fact, many of the results of the NHSLS replicate, albeit with a larger or more representative sample, results of these earlier studies.

The mass media have given extensive coverage to some of the results of the survey. Generally, articles and stories have focused on single numbers, such as the mean number of sexual partners. The results have typically been used to reinforce traditional values such as heterosexuality, monogamy, and marriage. According to *U.S. News* (17 Oct. 1994), "Fidelity reigns." The *U.S.A. Today* (7 Oct. 1994) headline read, "We are 'sexually conventional.' " The bottom line in many media treatments, and in *Sex in America*, is that sex is not nearly as frequent, exotic, or important as many people thought it was. On the one hand, this is a reassuring message to many whose sexual activities do not involve multiple orgasms and multiple partners. On the other hand, it is a highly selective interpretation. One could as readily focus on distributions, for example, the number of partners since age 18 ranges from 0 to over 1000, and emphasize the diversity of sexual expression in the contemporary United States.

On the whole, the NHSLS is a major accomplishment. Thanks to the extraordinary time and effort invested by the principals, we have comprehensive data on the sexual activities of a representative sample of Americans 18 to 59. These data provide a baseline against which the results of future studies will be compared. The focus on and analysis of sexual networks, and the analysis of unions and their characteristics are innovative. The publication of these books has focused attention on the validity of self-report data, which may lead to some methodological advances. The major strength of the research is its major limitation as well; it is a quantitative survey. Surveys cannot assess the cognitive and emotional processes or the dynamics of social interaction that lead to sexual expression. What is needed now are equally well-done qualitative studies of these processes.

John DeLamater
Center for Demography and Ecology, University of Wisconsin, Madison, WI 53706, USA



Scale in Ecology

Species Diversity in Space and Time. MICHAEL L. ROSENZWEIG. Cambridge University Press, New York, 1995. xxii, 436 pp., illus. $74.95 or £50; paper, $27.95 or £17.95.

Macroecology. JAMES H. BROWN. University of Chicago Press, Chicago, 1995. xiv, 269 pp., illus. $42.50 or £33.95; paper, $15.95 or £12.75.

When Robert MacArthur introduced the assembled students in his lectures on biogeography at Princeton in 1966 to the study of patterns in the number of species of plants and animals, I felt the thrill of bringing order to the bewildering variety that had drawn me to natural history in the first place. In his quiet yet deliberate manner, MacArthur showed how a broadly comparative approach coupled with the application of simple mathematical models could transform the ecological study of diversity from a mere cataloging of species and place names into the recognition of principles with explanatory power.

We have come a long way in our understanding of diversity since 1966, but the insights and enthusiasm that MacArthur imparted live on undiminished in Rosenzweig's wide-ranging treatise on the geography of diversity. In what may be the most important book on this subject since MacArthur's *Geographical Ecology* (Harper and Row, 1972), Rosenzweig combines theory with a huge body of empirical observations on terrestrial, aquatic, and marine organisms living today as well as in the geological past to produce a coherent account that brings together several previously separate research traditions ranging from experimental ecology to ecosystems analysis, paleontology, biogeography, and macroevolution. He not only reviews and summarizes the contribution of these fields but reanalyzes and reinterprets them, throughout emphasizing new approaches and new questions. Rosenzweig confronts the complexity of diversity directly, convincing the reader that a predictive understanding can come about only when we study the phenomenon at all scales of space and time. Why are there more species in the tropics than at higher latitudes? Why is polyploidy among plants more common on tropical mountaintops than in tropical lowlands, when in the temperate zones the proportions remain constant with altitude? Why are there so many rodents in earthquake-prone regions of the former Soviet Union, or so many plant species in the superficially monotonous South African fynbos or the southwest Australian kwongan heathlands?

More clearly than anyone else, Rosenzweig shows why we cannot employ small-scale patterns of diversity uncritically as models to explain larger scale patterns of diversity among biogeographical provinces and over geological time. When ecologists compare numbers of species among experimentally manipulated plots or even among islands in an archipelago, they are subsampling a known and relatively constant species pool. In comparisons among provinces, across latitudes, or over time, however, the pool of available species changes by virtue of such evolutionary processes as speciation, extinction, and large-scale invasion. Thus, although area emerges as the most important factor controlling diversity at all spatial scales of analysis, its precise relationship to species number differs strongly at the various scales. Productivity, or at least the access that organisms have to available energy and nutrients, is clearly also important, but here our understanding remains sketchy.

Brown's *Macroecology* covers some of the same ground but deals with a narrower range of scales—regional to global in space, decadal to millennial in time. With most of his data coming from the abundance, distribution, and sizes of North American mammals and birds, Brown's empirical base is much more limited than Rosenzweig's, and he runs the risk of wringing too much out of what may be a skewed sample of the biota. For example, given that nearly all large mammals and many large birds disappeared in North America at the end of the Pleistocene, how should we interpret the relationships among body size, abundance, and size of range that Brown documents for the living subsample of North American birds and mammals?

Nevertheless, Brown makes many important points that ecologists should ponder. Like Leigh Van Valen, whose important but often overlooked paper on energy (*Evol. Theory* 1, 179–229 [1976]) he cites, Brown argues that energy should be adopted as the preferred currency of ecological and evolutionary studies. He also persuasively pleads for the use of a diversity of methods in comparative biology, not only those derived from cladistic analysis as some biogeographers would insist. Brown recognizes that geographical range limits are changeable, that a given geographical configuration and geological history do not affect all lineages in the same way, and that many explanations typically considered mutually exclusive are instead complementary.

Both books go far in bridging the enormous gulf that has existed for decades between Big Ecology—the analysis of energy flow and nutrient cycles in ecosystems—and Little Ecology, the experimental dissection of the effects of competition, predation, host-guest relationships, and physical factors on local patterns in the abundance

of species. Anyone who doubts that these two traditions can be merged, or that comparative biology has a great deal to contribute to the emerging science of diversity, should read these books.

Geerat J. Vermeij
Department of Geology and Center of Population Biology, University of California, Davis, CA 95616, USA

■

Reprints of Books Previously Reviewed

Enrico Fermi, Physicist. Emilio Segrè. University of Chicago Press, Chicago, 1995. Paper, $13.95 or £11.25. *Reviewed* **169**, 846 (1970).

The Firecracker Boys. Dan O'Neill. St. Martin's Griffin, New York, 1995. Paper, $14.95 or $C21. *Reviewed* **266**, 663 (1994).

The Physics of Immortality. Frank J. Tipler. Anchor (Doubleday), New York, 1995. Paper, $14.95 or $C19.95. *Reviewed* **267**, 1042 (1995).

Unusual Telescopes. Peter Manly. Cambridge University Press, New York, 1995. Paper, $19.95. *Reviewed* **257**, 421 (1992).

■

Books Received

Antibody Engineering Protocols. Sudhir Paul, Ed. Humana, Totowa, NJ, 1995. xii, 450 pp., illus. Spiralbound, $69.50. Methods in Molecular Biology, 51.

The Belle Epoque of Surgery. The Life and Times of Theodor Billroth. Karel B. Absolon. Kabel, Rockville, MD, 1995. 214 pp., illus., + plates. $49.50; paper, $39.50.

Biochemistry. Lubert Stryer. 4th ed. Freeman, New York, 1995. xxxvi, 1064 pp., illus. $69.95.

Bluebirds and Their Survival. Wayne H. Davis and Philippe Roca. University Press of Kentucky, Lexington, 1995. x, 154 pp., illus. Paper, $15.95.

Changing the Rules. Psychology in the Netherlands, 1900-1985. Trudy Dehue. Cambridge University Press, New York, 1995. x, 204 pp., illus. $49.95. Cambridge Studies in the History of Psychology. Translated from the Dutch edition (Amsterdam, 1990) by Michael O'Loughlin.

Chaos, Gaia, Eros. A Chaos Pioneer Uncovers the Three Great Streams of History. Ralph H. Abraham. HarperSanFrancisco, San Francisco, 1994. xvi, 263 pp., illus. Paper, $16.

Chemistry of Structure-Function Relationships in Cheese. Edyth L. Malin and Michael H. Tunick, Eds. Plenum, New York, 1995. x, 397 pp., illus. $95. Advances in Experimental Medicine and Biology, vol. 367. From a symposium, Chicago, IL, Aug. 1993.

Children of the Atomic Bomb. An American Physician's Memoir of Nagasaki, Hiroshima, and the Marshall Islands. James N. Yamazaki, with Louis B. Fleming. Duke University Press, Durham, NC, 1995. xvi, 184 pp., illus. $16.95. Asia-Pacific: Culture, Politics, and Society.

Cycles in Humans and Nature. An Annotated Bibliography. John T. Burns. Scarecrow, Metuchen, NJ, 1994. xii, 288 pp. $37.50. Magill Bibliographies.

The Encyclopedia of Advanced Materials. David Bloor *et al.*, Eds. Pergamon (Elsevier Science), Tarrytown, NY, 1994. 4 vols. lii, 3152 pp., illus. $1600.

An Encyclopedia of Claims, Frauds, and Hoaxes of the Occult and Supernatural. James Randi. St. Martin's, New York, 1995. xx, 284 pp., illus. $24.95.

Essential Substances. A Cultural History of Intoxicants in Society. Richard Rudgley. Kodansha Globe, New York, 1995. x, 197 pp., illus. Paper, $12. Reprint, 1994 ed.

Essentials of Psychology in Action. Karen Huffman, Mark Vernoy, and Judith Vernoy. Wiley, New York, 1994. xxx, 522 pp., illus., + supplementary material. Paper, $29.50.

Ethics on the Ark. Zoos, Animal Welfare, and Wildlife Conservation. Bryan G. Norton *et al.*, Eds. Smithsonian Institution Press, Washington, DC, 1995. xxviii, 330 pp., illus. $32.50. Zoo and Aquarium Biology and Conservation. From a workshop, Atlanta, March 1992.

The Evening Star. Venus Observed. Henry S. F. Cooper, Jr. Johns Hopkins University Press, Baltimore, 1994. xiv, 300 pp., illus. Paper, $12.95. Reprint, 1993 ed.

Global Environmental Change. Interactions of Science, Policy, and Politics in the United States. Robert G. Fleagle. Praeger, Westport, CT, 1994. xvi, 243 pp. $59.95.

A Global Geochemical Database. For Environmental and Resource Management. A. G. Darnley *et al.* UNESCO, Paris, 1995. x, 122 pp., illus. Paper. Earth Sciences, 19. Final Report of IGCP Project 259.

The Global Geospace Mission. C. T. Russell, Ed. Kluwer, Norwell, MA, 1995. viii, 877 pp., illus. $349 or £209 or Dfl. 495. Reprinted from *Space Science Reviews*, vol. 71, nos. 1-4, 1995.

Hochschild Cohomology of von Neumann Algebras. Allan M. Sinclair and Roger R. Smith. Cambridge University Press, New York, 1995. viii, 196 pp. Paper, $29.95. London Mathematical Society Lecture Note, 203.

Hormonal Regulation of Bone Mineral Metabolism. Daniel D. Bikle and Andres Negro-Vilar, Eds. Endocrine Society Press, Bethesda, MD, 1995. vi, 397 pp., illus. Paper, $60; to society members, $50. Endocrine Reviews Monographs, 4.

Hot Hadronic Matter. Theory and Experiment. Jean Letessier, Hans H. Gutbrod, and Johann Rafelski, Eds. Plenum, New York, 1995. xii, 562 pp., illus. $145. NATO ASI Series B, vol. 346.

Intercellular Signalling in the Mammary Gland. Colin J. Wilde, Malcolm Peaker, and Christopher H. Knight, Eds. Plenum, New York, 1995. xii, 296 pp., illus. $89.50. From a symposium, Ayr, UK, April 1994.

International Handbook of Personality and Intelligence. Donald H. Saklofske and Moshe Seidner, Eds. Plenum, New York, 1995. xxiv, 776 pp., illus. $95. Perspectives on Individual Differences.

International Tables for Crystallography. Vol. A, Space-Group Symmetry. Theo Hahn, Ed. 4th ed. Published for the International Union of Crystallography by Kluwer, Norwell, MA, 1995. xvi, 878 pp., illus. $180 or £120 or Dfl. 340.

Interstitium, Connective Tissue and Lymphatics. R. K. Reed *et al.*, Eds. Portland, London, 1995 (U.S. distributor, Ashgate, Brookfield, VT). xiv, 341 pp., illus. $120 or £75. Proceedings, 9. From a congress, Glasgow, UK, 1993.

Memory's Ghost. The Strange Tale of Mr. M. and the Nature of Memory. Philip J. Hilts. Simon and Schuster, New York, 1995. 253 pp. $22.

Microbiology and Immunology. An Illustrated Review with Questions and Explanations. David J. Hentges. 2nd ed. Little Brown, New York, 1995. xvi, 288 pp., illus. Paper, $27.95.

Modern Practice of Gas Chromatography. Robert L. Grob. 3rd ed. Wiley, New York, 1995. xiv, 888 pp., illus. $89.95.

Modern Synthetic Methods 1995. Beat Ernst and Christian Leumann, Eds. Verlag Helvetica Chimica Acta, Basel, Switzerland, and VCH, New York, 1995. x, 453 pp., illus. $80.

Modified Lipoproteins in the Pathogenesis of Atherosclerosis. Sampath Parthasarathy. Landes, Georgetown, TX, 1994 (distributor, CRC Press, Boca Raton, FL). x, 131 pp., illus. $89.95. Medical Intelligence Unit.

The Periodic Kingdom. A Journey into the Land of the Chemical Elements. P. W. Atkins. BasicBooks, New York, 1995. xii, 163 pp., illus. $20. Science Masters.

Personal Liberty and Community Safety. Pretrial Release in the Criminal Court. John S. Goldkamp *et al.* Plenum, New York, 1995. xxiv, 342 pp., illus. $45. Plenum Series in Crime and Justice.

Petroleum Sedimentology. Winifred Zimmerle. Kluwer, Norwell, MA, 1995. x, 413 pp., illus. $169 or £110 or Dfl. 260.

Photonic Crystals. Molding the Flow of Light. John D. Joannopoulos, Robert D. Meade, and Joshua N. Winn. Princeton University Press, Princeton, NJ, 1995. xii, 137 pp. $35 or £30.

The Physiology of Fungal Nutrition. D. H. Jennings. Cambridge University Press, New York, 1995. xvi, 622 pp., illus. $150.

Picturing Health and Illness. Images of Identity and Difference. Sander L. Gilman. Johns Hopkins University Press, Baltimore, 1995. 200 pp., illus. $32.95. Published in UK by Reaktion Books as *Health and Illness: Images of Difference*.

Population Management for Survival and Recovery. Analytical Methods and Strategies in Small Population Conservation. J. D. Ballou, M. Gilpin, and T. J. Foose, Eds. Columbia University Press, New York, 1995. xx, 375 pp., illus. Paper, $29.50 or £21. Methods and Cases in Conservation Science.

A Primer of Conservation Biology. Richard B. Primack. Sinauer, Sunderland, MA, 1995. x, 278 pp., illus. Paper, $18.95.

Principles of Biochemistry. Geoffrey L. Zubey, William W. Parson, and Dennis E. Vance. Brown, Dubuque, IA, 1995. xxxii, 864 pp., illus., + supplementary material. $80.11.

Principles of Plasma Discharges and Materials Processing. Michael A. Lieberman and Allan J. Lichtenberg. Wiley, New York, 1994. xxvi, 572 pp., illus. $54.95.

Quantum Measurement. Vladimir B. Braginsky and Farid Ya. Khalili. Cambridge University Press, New York, 1995. xviii, 193 pp. Paper, $24.95. Reprint, 1992 ed.

The Search for God. Can Science Help? John Houghton. Lion, Oxford, UK, 1995. 224 pp., illus. Paper, £6.99.

Sedimentographica. Photographic Atlas of Sedimentary Structures. Franco Ricci Lucchi. 2nd ed. Columbia University Press, New York, 1995. x, 255 pp., illus. $45 or £31.50. Translated from the Italian edition (Bologna, 1992).

Self-Motion. From Aristotle to Newton. Mary Louise Gill and James G. Lennox, Eds. Princeton University Press, Princeton, NJ, 1994. xxii, 367 pp. $45 or £36.50. Based on a conference, Pittsburgh, Feb. 1990.

The Seven Thunders of the Soul. A Unified General Theory of Behavior. Henry Jacobs. Trinity Research Center, Ashland, MS, 1995. vi, 162 pp., illus. Paper, $14.95.

Sexuality. A Developmental Approach to Problems. Betty N. Gordon and Carolyn S. Schroder. Plenum, New York, 1995. xviii, 157 pp. $35. Clinical Child Psychology Library.

The Shoulders of Giants. A History of Human Flight to 1919. Phil Scott. Addison-Wesley, Reading, MA, 1995. xiv, 337 pp., illus., + plates. $24.

Single-Channel Recording. Bert Sakmann and Erwin Neher, Eds. 2nd ed. Plenum, New York, 1995. xxiv, 700 pp., illus., + plates. $89.50.

The Social and Interactional Dimension of Human-Computer Interfaces. Peter J. Thomas, Ed. Cambridge University Press, New York, 1995. x, 268 pp., illus. $54.95. Cambridge Series on Human-Computer Interaction, 9.

Publishers' Addresses

Below is information about how to direct orders for books reviewed in this issue. A fuller list of addresses of publishers represented in *Science* appears in the issue of 26 May 1995, page 1220.

Cambridge University Press, 110 Midland Ave., Port Chester, NY 10573–4930. Phone: 800-872-7423; 916-937-9600. Fax: 914-937-4712.

Little, Brown and Co., 200 West St., Waltham, MA 02154. Phone: 800-759-0190; 617-890-2125. Fax: 617-890-0875.

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Warner Books, P.O. Box 690, Special Sales Dept., 1217 Avenue of the Americas, New York, NY 10020. Phone: 800-222-6747; 212-522-6982. Fax: 800-222-6902.

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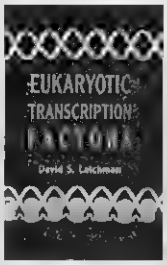

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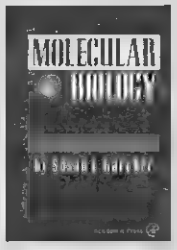

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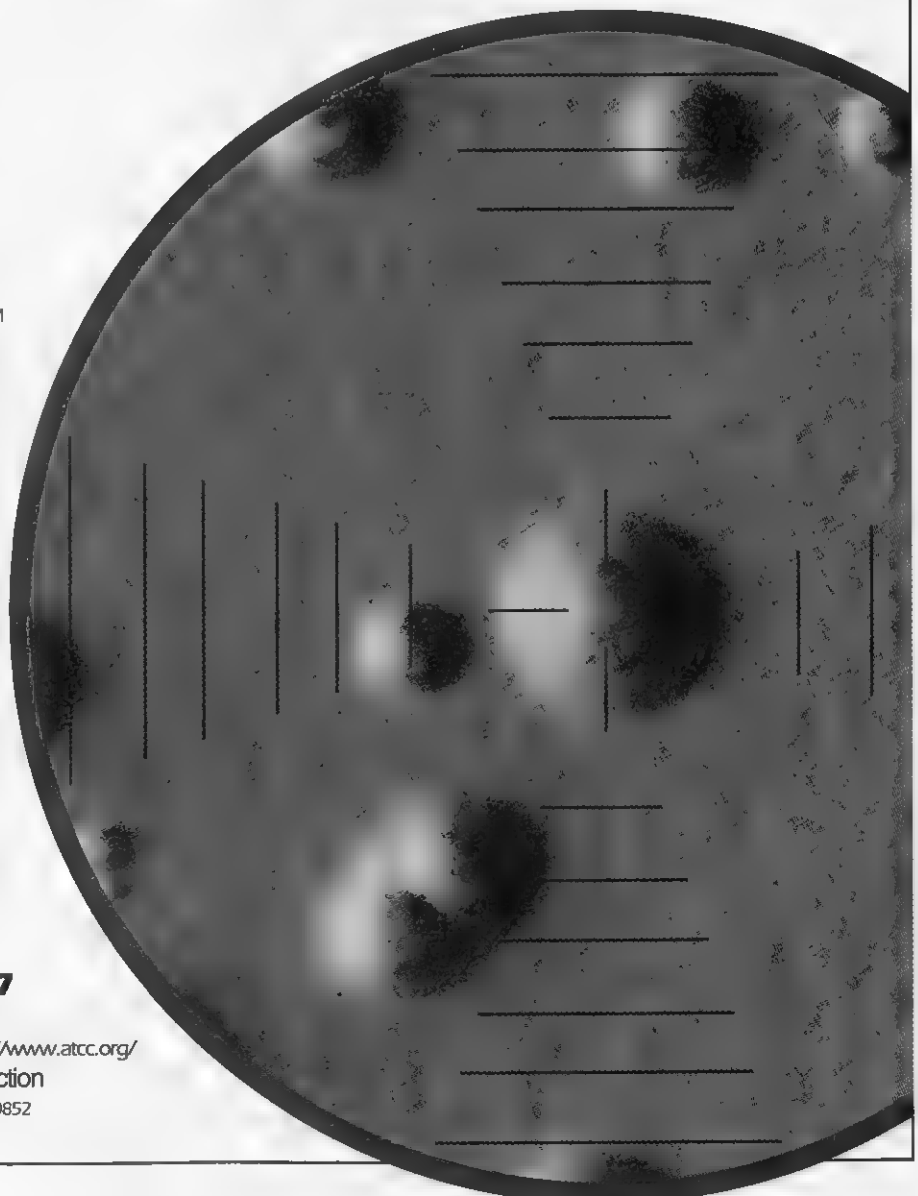

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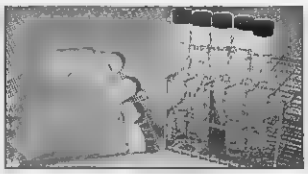

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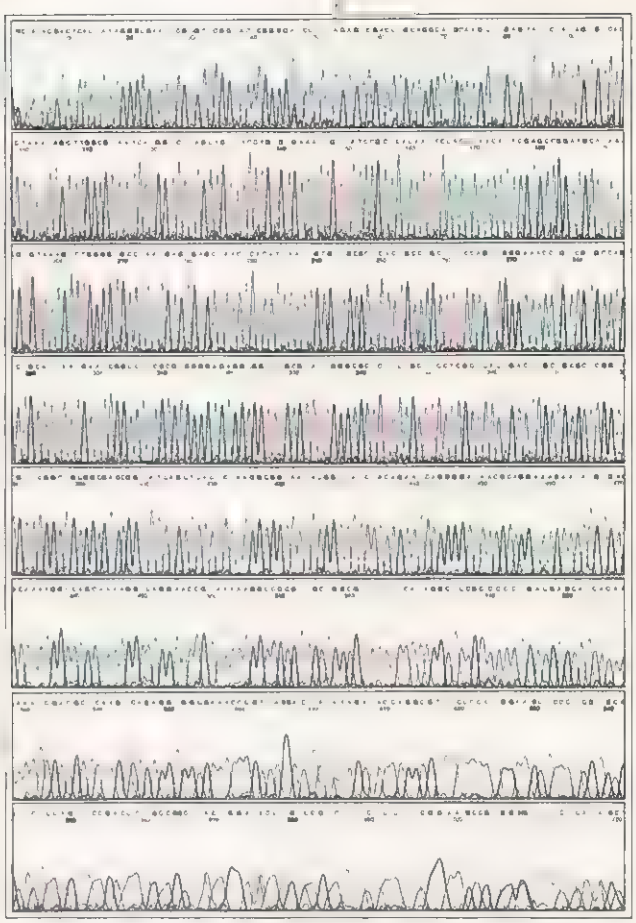
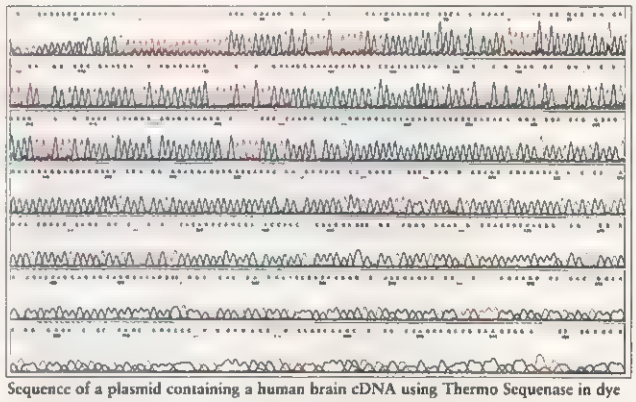


Cycle sequencing of pGEM plasmid using Thermo Sequenase fluorescent labelled primer cycle sequencing kit.
(Data supplied by an independent research laboratory)



Sequence of a plasmid containing a human brain cDNA using Thermo Sequenase in dye primer cycle sequencing.
(Data supplied by Généthon, 1 rue de l'International, 91002, Evry, France)

THERMO Sequenase

1. *Proc. Natl. Acad. Sci.,USA*, 92(14), pp 6339-6343, (1995).

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* Patent pending

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The sequence databases depend on scientists to contribute sequences and associated biological annotation in electronic form. The three databases in the U.S., Europe, and Japan collaborate in collecting and distributing all sequence information received and consequently make available comprehensive collections of DNA and RNA sequence data in the GenBank, EMBL, and DDBJ databases.

Researchers are encouraged to submit data to whichever of the three databases is most convenient. The receiving database will transmit the data to the other two. Collectively, the databases make the sequence information available to scientists throughout the world through the Internet and CD-ROM subscriptions.

Many editorial policies require submission of sequence data and the issuance of an accession number by the databases as a condition of publication. (At the authors' option, sequence data may be kept confidential until publication.) Including the accession number in publications makes it easy for users to retrieve the sequence from the databases. Sequence data can be submitted using free software for PC and Macintosh computers or through the World Wide Web (WWW). For assistance with sequence submission, contact the most convenient database:

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Telephone:	+1 (301) 496-2475	+44 (0)1223 494400	+81 559 81 6853
Telefax:	+1 (301) 480-9241	+44 (0)1223 494472	+81 559 81 6849
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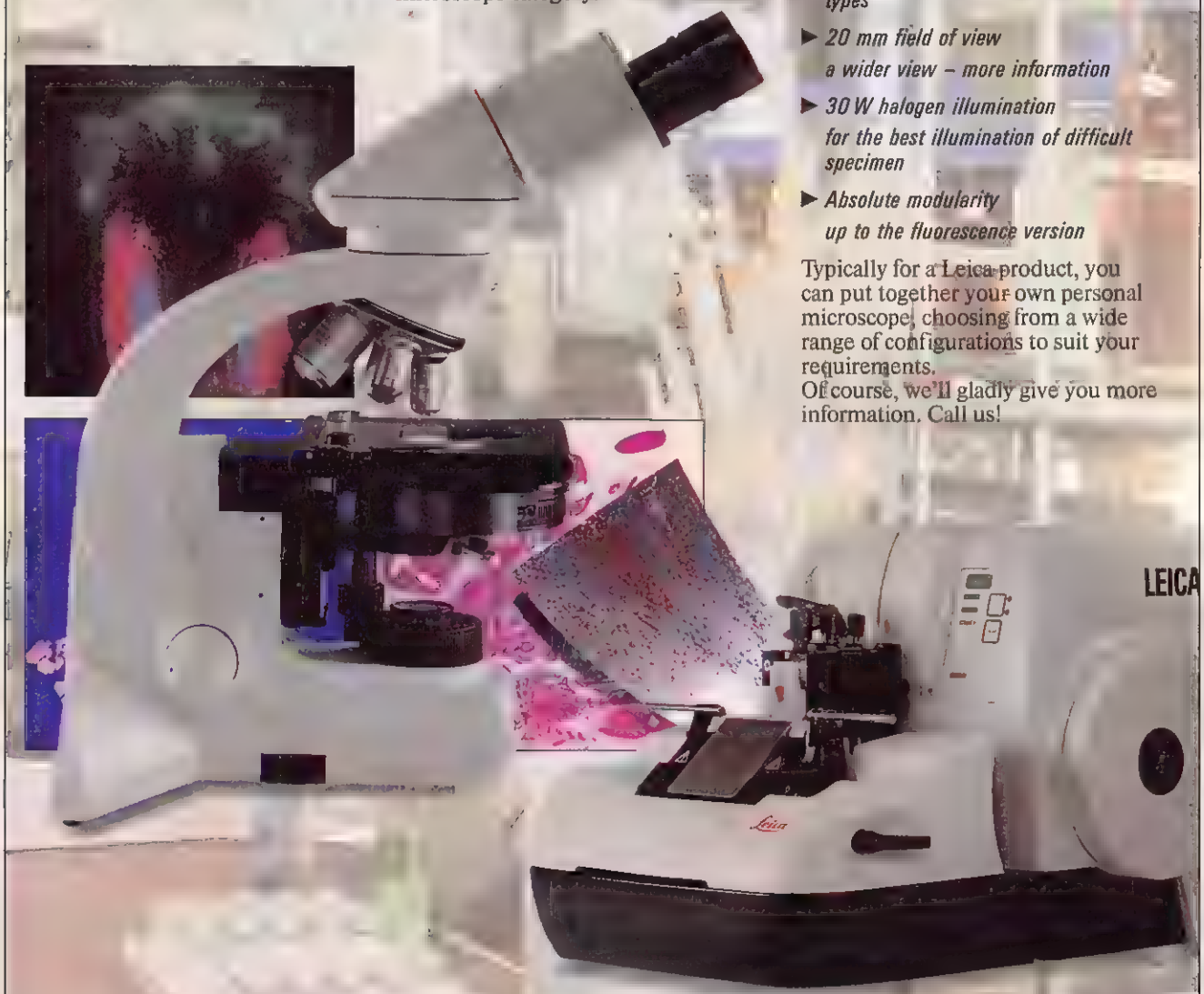

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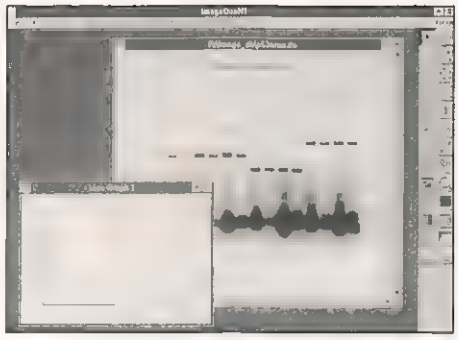

FluorImager SI, the assays require no film exposures or darkroom processing. **Vistra Systems. Circle 137.**

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A mAb is available to the recently described human activated lymphocyte cellular adhesion molecule. **Antigenix America. Circle 141.**

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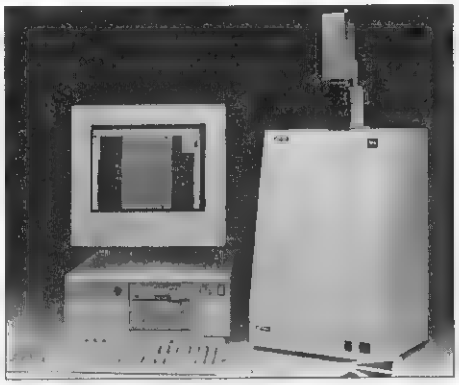

archiving on the user's hard drive. There is no need to take trial and error shots with Polaroid film—Gel Doc can work with any Macintosh- or Windows-compatible printer. The system consists of a combined transilluminator and fluorescent darkroom with a high-resolution charge-coupled device camera and Windows, Macintosh, or Power Macintosh software. The unit measures just 34 cm by 24 cm by 63 cm. **Bio-Rad Laboratories. Circle 145.**

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Literature

Growth Factors and Cytokines describes a line of these products. **Sigma Biosciences. Circle 146.**

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TIGR HCD
A New Resource for Genomics Research.

The Institute for Genomic Research is making its Human cDNA Database (TIGR HCD) of more than 345,000 complementary DNA (cDNA) sequences (and certain associated clones) available to scientists conducting basic research at non-profit institutions. These data were the basis for the paper, "Initial assessment of human gene diversity and expression patterns based upon 83 million nucleotides of cDNA sequence,"[1] published in *Nature* on September 28, 1995.

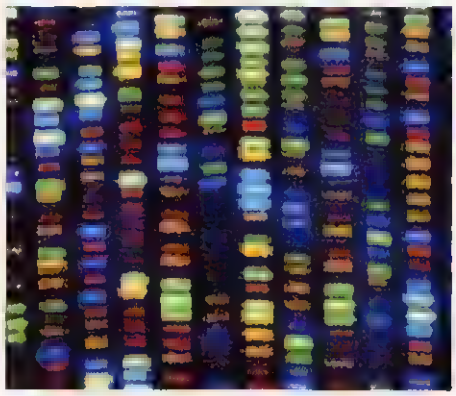
TIGR HCD is a curated relational database of cDNA fragments and assemblies, including tissue-specific expression data and (where possible) positive or tentative gene identifications.

The Institute for Genomic Research created the HCD — with scientific and financial support from Human Genome Sciences (HGS) and SmithKline Beecham (SB) — to offer researchers Internet access (via e-mail and the World Wide Web) to sequence and related data generated by privately financed studies carried out at TIGR and HGS[2].

Access •There is no charge for access to the TIGR Human cDNA Database. •Clones for TIGR sequences are available from the American Type Culture Collection at a nominal charge. •TIGR HCD is open to employees of non-profit or governmental orgnizations. •There are no restrictions on access to some 300,000 EST sequences substantially identical to whole or partial gene sequences already published. •An additional 45,000 unpublished ESTs and assemblies (and associated clones) are available under terms that reflect provisions common in academic and industrial material transfer agreements.

Queries: TIGR HCD users can search the database by protein or gene name (putative identification), by nucleotide or peptide sequence, or by TIGR accession number (as listed in reference 1). Results can range from a list of entries with specified similarity to full sequences of matching entries.

Data: The data in HCD have been generated by the expressed sequence tag (EST) method, which provides rapid characterization of expressed genes by partial DNA sequencing[3]. HCD data include nucleotide sequences, putative sequence identifications, and tissue-based expression information derived from a large-scale EST survey of human tissues. The data set includes 40,000 assemblies, constructed by combining overlapping ESTs. And all nucleotide data are searched in six-frame translation — that is, the database searches all reading frames for both the sequence and its antisense strand.



For more information regarding TIGR HCD (including user application forms), or contact the TIGR Database Manager at:

e-mail: info@hcd.tigr.org
fax: (301) 838-0218
mail: Manager, TIGR Database
The Institute for Genomic Research
9712 Medical Center Drive
Rockville, MD 20850

world wide web: http://www.tigr.org(TIGR Database)

[1] *Adams MD, et al. 1995. Nature, 377:250-330*

[2] *Of TIGR HCD's 345,000 expressed sequence tags, approximately 105,000 were generated at TIGR and 55,000 at HGS; the remainder derive from sources in the public domain.*

[3] *Adams MD, et al. 1991. Complementary DNA sequencing; Expressed sequence tags and the Human Genome Project. Science, 252:1651-1656*

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The Department of Microbiology at the University of Tennessee, Knoxville, invites applications for a tenure track faculty appointment as **ASSISTANT PROFESSOR**. Candidates for the position should have a commitment to applying modern molecular techniques in the analysis of microbial populations and interactions in biogeochemical processes. The University community and the nearby facilities of Industry, Oak Ridge National Laboratory, National Park Service, and the Tennessee Valley Authority offer unique opportunities for collaborative investigations. Preference will be given to applicants with a willingness to become part of this interacting network. The successful applicant must hold a doctoral degree with at least two years of postdoctoral experience in modern molecularly based Environmental Microbiology and Microbial Ecology, demonstrated research productivity, and evidence of potential for excellence in teaching. Responsibilities include graduate and undergraduate teaching in Environmental Microbiology or Microbial Ecology, establishment of a productive, independent research program, and academic responsibilities normally associated with a tenure-track faculty position. The starting date is August 1996.

Applicants should submit a letter of interest accompanied by curriculum vitae, a description of research interests and career objectives, and the names, addresses and telephone numbers of three individuals who can evaluate the applicant's qualifications for the position. All material should be sent to: **Dr. Gary S. Sayler, Chair of Search Committee, Department of Microbiology, The University of Tennessee, Knoxville, TN 37996-0845. Telephone: 423-974-8080** for additional information. Nominations should be made as soon as possible: evaluation of applications will begin in January 1996. *The University of Tennessee is an Equal Employment Opportunity/Affirmative Action/Title IX/Section 504/ADA Employer. Applications from women and minorities are especially invited.*

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Screening of applicants will begin December 1, 1995, and continue until the position is filled. *University of Missouri-Kansas City is an Equal Opportunity/Affirmative Action Employer.*

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2) Quantitative Ecologist. Must have expertise in application of advanced statistical methods in ecological research and experience in a field of ecology that will complement existing programs. Teaching to include participation in lower division course in biostatistics, and upper division/graduate courses in advanced statistics, sampling design, ecological modeling, or other courses relating to ecological specialty. Should have good communication skills and strong interest in collaborating with ecology colleagues and working with graduate students. Evidence of research productivity is essential. More information on both positions available on internet at **http://www.sci.sdsu.edu/fac-recruitment.** Closing date for receipt of applications is November 21, 1995. Applicants for either position should submit a curriculum vitae, research/teaching statement, names and addresses of three references to: **Evolutionary Biology Search Committee** or **Quantitative Ecology Search Committee, Department of Biology, San Diego State University, San Diego, CA 92182.** *San Diego State University is an Affirmative Action/Equal Opportunity/Title IX Employer.*

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Ecologist Search Committee
Department of Biology
Campus Box 1137
Washington University
1 Brookings Drive
St. Louis, MO 63130-4899

All applications should be received by December 1, 1995. *Washington University is an Affirmative Action/Equal Opportunity Employer.*

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One applicant should be prepared to teach courses in botany, an advanced field biology course in his or her area of specialization, and alternate teaching of the freshman introductory biology course. A small field station is located near the campus, and opportunities for field work with various governmental agencies are available.

One applicant should be prepared to teach cell/molecular biology, genetics, and an advanced course in his or her area of specialization.

Send application, curriculum vitae, transcripts, and the names, addresses and telephone numbers of three references to: **Dr. Elaine Chapman, Department of Biology, Illinois College, Jacksonville, IL 62650.** Screening of applications will begin November 1, 1995. *Illinois College is an Affirmative Action/Equal Opportunity Employer.*

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Department of Genetics/Molecular Medicine
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Douglas C. Wallace, Ph.D.
Robert W. Woodruff Professor of Molecular Genetics
Chairman, Department of Genetics
and Molecular Medicine
1462 Clifton Road, Room 446
Emory University School of Medicine
Atlanta, GA 30322

Emory University is an Equal Opportunity/Affirmative Action Employer.

Biochemist/Williams College—The Chemistry Department invites applications for a tenure-eligible position at the **ASSISTANT PROFESSOR** level for fall 1996. (Senior appointment possible in exceptional circumstances.) This will be an initial appointment for a three-year term with teaching assignments in biochemistry and introductory chemistry. A semester teaching load normally includes complete responsibility for one course and two laboratory sections, and supervision of student research projects. Candidates should have the Ph.D. or completed dissertation by September 1996 (postdoctoral experience is preferred) and a strong commitment to teaching at the undergraduate level and developing a productive research program. Williams College is a highly selective, coeducational liberal arts institution of approximately 230 faculty and 2000 undergraduates, located in northwestern Massachusetts. The Chemistry Department, with ten faculty members and 30 to 35 majors each year, is accredited by the ACS and has excellent facilities for teaching and research. A new 40-million dollar science complex will be completed in the year 2000. *The College is actively working to increase the ethnic and gender diversity of its science majors and seeks an individual who can help us meet these goals.* Send résumé, undergraduate and graduate transcripts, description of research projects for undergraduates, and three letters of recommendation to: **Professor John W. Thoman, Jr., Chair, Department of Chemistry, Williams College, Williamstown, MA 01267**, by November 30, 1995. *Williams is an Equal Opportunity/Affirmative Action Employer.*

DEVELOPMENTAL GENETICS/BIOLOGY

The Department of Biology at the University of Central Florida invites applicants for a Tenure-Track Faculty position at the **ASSISTANT PROFESSOR** level starting August 1996. We are particularly interested in individuals using genetic and molecular approaches to understanding mechanisms of development in animal systems. A Ph.D. and postdoctoral experience or equivalent are required at the time of application. The successful candidate will be expected to have a productive research program that will gain extramural funding, and to participate in undergraduate and graduate teaching in Genetics and Developmental Biology. Candidates should submit a curriculum vitae, the names and addresses of three references, and statements of research and teaching interests. Screening will begin December 15, 1995. Send material to: **David T. Kuhn, Search Committee Chair, Department of Biology, University of Central Florida, Orlando, FL 32816.** *The University of Central Florida is an Equal Opportunity/Affirmative Action Employer.* As an agency of the State of Florida, all application materials and selection procedures are available for public review.

POSITIONS OPEN

INSECT SYSTEMATIST

The Department of Entomology, University of Kentucky invites applications for a 12-month, tenure-track **ASSISTANT PROFESSOR** of Entomology (85% research, 15% teaching) position studying insect systematics. Ph.D. in Entomology or Biological Sciences required; postdoctoral experience and evidence of successful funding preferred. We are searching for a modern insect systematist who works on economically important insects. Research topics might include, but are not limited to, phylogenetic studies, comparative studies and systematics of the morphology, ecology, behavior or genetics of beneficial or pest insect species. Use of molecular techniques is preferred. The successful applicant will be expected to develop and maintain a vigorous research program, compete for extramural funding, train M.S. and Ph.D. students, teach Insect Taxonomy, and develop a graduate-level course in the applicant's area of expertise. Send letter of application, résumé, transcripts of all university work, statement of research and teaching interest and philosophy, a copy of reprints, and request that four letters of reference be sent by February 15, 1996 to: **Dr. B. C. Pass, Chair, Department of Entomology, University of Kentucky, Lexington, KY 40546-0091.** *The University of Kentucky is an Equal Opportunity/Affirmative Action Employer.*

ANIMAL PHYSIOLOGIST

The Biology Department, Oberlin College, invites applications for a tenure-track faculty appointment to begin fall 1996. Although preference will be given to candidates at the rank of **ASSISTANT PROFESSOR**, outstanding applicants at the Associate Professor rank will also be considered. We seek a broadly trained animal physiologist who uses modern experimental methods to address questions at the organ/systems level. The successful candidate will be expected to teach an upper-level animal physiology course with laboratory, and a second upper-level course with laboratory in her or his area of interest; contribute to the teaching at the introductory level; and supervise individual undergraduate student research projects. He or she will also be expected to participate in the full range of faculty responsibilities including academic advising of undergraduates; serving on committees; and sustaining an active research program. A strong interest and potential excellence in teaching at the undergraduate level is essential. Postdoctoral experience is desirable. Send curriculum vitae, graduate transcript, and three letters of recommendation to: **Dr. Dennis N. Luck, Chair, Department of Biology, Oberlin College, Oberlin, OH 44074.** Review of applications will begin December 1, 1995 and continue until the position is filled. *Oberlin College admitted women since its beginnings in 1833, and has been historically a leader in the education of blacks. Affirmative Action/Equal Opportunity Employer.*

BIOLOGY/MYCOLOGY

ASSISTANT PROFESSOR. TENURE-TRACK. Ph.D. required. Broadly-trained mycologist with strong commitment to undergraduate teaching. Expectations: teach undergraduate and graduate mycology courses, including field courses and courses in area of expertise; participate in large general biology program; serve as mentor for undergraduate research and Master's theses; seek extramural funding. Send letter of application; curriculum vitae; description of research interests; names, addresses, and telephone numbers of three references to: **Dr. Douglas Meikle, Chair, Department of Biology, Appalachian State University, 572 Rivers Street, Boone, NC 28608. Telephone: 704-262-3025.** Completed applications due by December 1, 1995. *Appalachian State University is an Equal Opportunity Employer.*

ASSISTANT PROFESSOR OF BIOLOGY

Tenure-track fall 1996. Expertise in zoology and ecology to teach introductory zoology, participate in non-major course offerings, and teach upper-level courses in ecology and animal physiology or in an area of specialty. Ph.D. required, teaching experience preferred, must have interest in providing quality undergraduate instruction at a church-related college. Deadline for letter of application, official undergraduate and graduate transcripts, curriculum vitae, and names, addresses, and telephone numbers of three references is November 20, 1995. Send to: **Biology Search Committee, Personnel Office, Whitworth College, Spokane, WA 99251.** *Whitworth College strongly encourages females, persons of color, and persons with physical disabilities.*

POSITIONS OPEN

VERTEBRATE PHYSIOLOGIST
Department of Organismic and Evolutionary Biology
Harvard University

The Department seeks a vertebrate physiologist who employs comparative, integrative approaches to address major problems in whole organism physiology. The appointment will be to a tenured **PROFESSORSHIP**, and the incumbent will be expected to assume the Directorship of the Concord Field Station of the Museum of Comparative Zoology. We are especially interested in individuals whose research experience and interests are based on integrative approaches to understanding the diversity of form and function across major taxonomic groups. Applicants should submit by 20 November 1995 a curriculum vitae, a statement of research and teaching interests, and the names and addresses of three references to:

Professor Farish A. Jenkins, Jr.
Chairman, Vertebrate Physiology Search Committee
Department of Organismic and Evolutionary Biology
Harvard University
26 Oxford Street
Cambridge, MA 02138

Harvard University is an Affirmative Action/Equal Opportunity Employer. We encourage applications from women and minority groups.

PLANT MOLECULAR SYSTEMATIST
University of Maryland, College Park

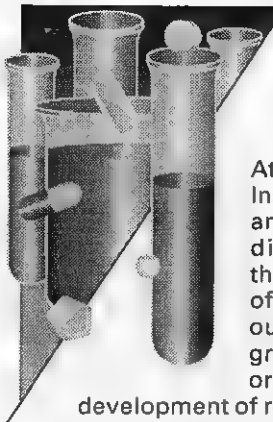
The Department of Plant Biology invites applications and nominations for a tenure-track position, preferably at the rank of **ASSISTANT PROFESSOR**, to begin by September 1996. Candidates must have a Ph.D. (or equivalent) in biological sciences and a strong knowledge of plant diversity. The successful candidate is expected to complement existing programs in ecology and evolutionary biology and to establish an outstanding, externally funded research program using modern methods of molecular biology and computer analysis to resolve fundamental systematic problems for plants in an evolutionary context. Teaching responsibilities include a general course in biology and an advanced course in molecular systematics. To apply, please submit a curriculum vitae, copies of published and in-press works, a description of current and projected research interests, a summary of teaching interests and experiences, and names and addresses (including Email addresses) of four references to: **Chair, Molecular Systematist Search, Department of Plant Biology, University of Maryland, College Park, MD 20742-5815.** For best consideration, applications should be received by December 15, 1995. *The University of Maryland is an Equal Employment Opportunity/Affirmative Action Employer. Women, minorities, and persons with disabilities are encouraged to apply.*

BEHAVIORAL ECOLOGIST

ASSISTANT PROFESSOR, tenure-track. Qualifications: Ph.D., commitment to teaching undergraduates in a liberal arts setting. Teach introductory biology, courses in the specialty, and develop a research program involving undergraduates. Send résumé, statement of research plans and undergraduate teaching philosophy, transcripts, and have three letters of reference sent to: **Dr. Gregg Kormanik, Chair, Department of Biology, University of North Carolina at Asheville, One University Heights, Asheville, NC 28804.** Review of applications will begin 1 December 1995. *University of North Carolina at Asheville is an Equal Opportunity Employer. Applications from underrepresented groups including minorities, women, and people with disabilities are encouraged.*

PLANT ECOLOGIST

Applications are invited for an **ASSISTANT PROFESSOR**, tenure-track position beginning September 1996. Ph.D. required; postdoctoral experience preferred. The applicant should be able to teach ecology, plant ecology, and plant taxonomy and develop a research program involving undergraduate and graduate students. Send curriculum vitae, copies of publications, and letters from three references to: **Dr. Carl Quertermus, Chair Search Committee, Biology Department, West Georgia College, Carrollton, GA 30118. Telephone: 770-836-6547. For position information see http://www.westga.edu/localhome.html.** Review of applications will begin December 1, 1995. *An Affirmative Action/Equal Opportunity Employer.*



POSTDOCTORAL FELLOWS

At The Glaxo Wellcome Research Institute, located in Research Triangle Park, N.C., the focus is drug discovery and development through novel research. As part of our commitment to research, our Postdoctoral Fellowship Programs support scientific endeavors and scholarships through the development of researchers at the early stage of their careers. We are currently seeking to fill three Postdoctoral Fellows in the following areas:

STRUCTURAL CHEMISTRY

We seek a recent Ph.D. in Analytical Chemistry or Biochemistry to perform research in deuterium exchange and mass spectrometry of proteins to examine higher order structure and interactions. Candidates with experience in mass spectrometry and protein biochemistry preferred. **Please refer to Job # ASC076401 on all resumes.**

MOLECULAR PHARMACOLOGY

Individual will investigate the role of orphan nuclear hormone receptors in the regulation of bone marrow cell differentiation. This will include an examination of the peroxisome proliferator activated receptors and their role in regulating marrow adipogenesis. Candidates should have a recent Ph.D. in Molecular Biology or related area and interest in transcriptional regulation. Experience in cell culture techniques required. **Please refer to Job # ASC076501 on all resumes.**

PHARMACOLOGY

Individual will isolate and characterize the function and regulation of intracellular proteins associated with the expression of intracellular calcium. Will design and conduct experiments to study this process in physiologic and pathophysiologic instances. Candidates must have a recent Ph.D. in Biochemical Pharmacology, Biochemistry, or Molecular Biology. Experience in protein isolation, purification, single cell isolation and culturing, and gene cloning preferred. **Please refer to Job # ASC076601 on all resumes.**

These Fellowship openings offer you the opportunity to strengthen your scientific reputation and career advancement by publication and presentation of outstanding research conducted in an excellent environment. In addition, Glaxo Wellcome is proud to offer a highly competitive compensation package. Applicants should send their curriculum vitae, a statement of research interest, a list of professional references, and the Job # of interest, to: **Human Resources Department, Glaxo Wellcome Research Institute, P.O. Box 13398, Research Triangle Park, NC 27709.** (No Phone Calls or Agency Referrals, Please.) An Equal Opportunity Employer M/F/D/V.

Glaxo Wellcome

National Institutes of Health / Public Health Service
Department of Health and Human Services

HEALTH SCIENTIST ADMINISTRATOR/MEDICAL OFFICER

OFFICE OF AIDS RESEARCH

The National Institutes of Health (NIH) invites applications for the position of Health Scientist Administrator/Medical Officer, Office of AIDS Research, Office of the Director. This is a Civil Service position with a salary ranging from the GS-14 to GS-15 pay level per annum (currently $60,925 to $93,166, including locality pay). Medical Officers may be eligible for a salary up to $97,386 plus a Physician's Comparability Allowance.

The Health Scientist Administrator/Medical Officer will serve as the Chair of the Therapeutics Coordinating Committee responsible for facilitating and coordinating efforts of the committee in strategic planning, identifying scientific opportunities, setting research priorities, and enhancing trans-NIH coordination and collaboration. The incumbent will analyze the recommendations made by major advisory committees and governmental oversight bodies for their impact on a diverse range of health care providers, researchers, advocacy groups, and the public, and will coordinate the implementation of these recommendations with the Institutes, Centers, and Divisions of NIH. The incumbent will identify issues and prepare position papers for conferences and workshops, prepare a variety of written materials used for congressional testimony and press conferences, serve on ad hoc task forces, and prepare speeches and informal remarks for the Associate Director for AIDS Research and other top-level NIH officials.

Applicants should submit either an Optional Application for Federal Employment (OF-612), or any other written format (including an SF-171 or resume); and a description of your accomplishments that demonstrate competence in the following Knowledge, Skills, and Abilities: (1) Knowledge of AIDS-related research in the study of Therapeutics; (2) Experience in preparing budgets and budget narratives; (3) Demonstrated skill in preparing verbal and written reports on complex scientific issues associated with the Therapeutics field; and (4) Experience in facilitating and coordinating committees with the goal of identifying scientific opportunities and setting research priorities. A complete application package may be obtained by calling (301) 402-4111 (vacancy announcement number OD-95-1084). The TDD number is (301) 402-1970. Application materials must be sent to the following address:

NATIONAL INSTITUTES OF HEALTH
OFFICE OF THE DIRECTOR
PERSONNEL OFFICE
ATTN: MS. GWEN CURRIE
BUILDING 31, ROOM 1C27
31 CENTER DR MSC 2264
BETHESDA MD 20892-2264

NATIONAL INSTITUTES OF HEALTH

Applications must be postmarked no later than January 2, 1996.

Selection for this position will be based solely on merit, without discrimination for non-merit reasons such as race, color, religion, gender, national origin, political affiliation, marital status, sexual orientation, physical or mental disability, age, or membership or non-membership in an employee organization.

NIH is an Equal Opportunity Employer

POSITIONS OPEN

TENURE-TRACK FACULTY POSITIONS
Department of Microbiology and Immunology
SUNY Health Science Center at Syracuse

The Department of Microbiology and Immunology at the SUNY Health Science Center at Syracuse invites applications for two tenure-track, full-time, positions at the **ASSISTANT** or **ASSOCIATE PROFESSOR** level. Competitive applicants will have a Ph.D. degree or equivalent, postdoctoral experience, and a record of innovative research productivity. Applicants for Associate Professor should have current research funding. Preference will be given to candidates with research interests in microbial pathogenesis, molecular virology, and gene therapy. Salary, space, and start-up funds are competitive with national levels. Applications including a statement of research interests and goals; a curriculum vitae; and names, addresses, and telephone numbers for three references should be sent to: **Steven M. Taffet, Ph.D., Chair of the Search Committee, Department of Microbiology and Immunology, SUNY Health Science Center at Syracuse, 750 East Adams Avenue, Syracuse, NY 13210.** The deadline for applications is December 15, 1995.

The SUNY Health Science Center at Syracuse is an Equal Opportunity/Affirmative Action Employer.

Rose Houchins Distinguished Professorship of Radiochemistry

Applications are invited for a **ROSE HOUCHINS DISTINGUISHED PROFESSORSHIP** of Radiochemistry in the Chemistry Department at the University of Missouri-Columbia. Appointment is anticipated for August, 1996. Special preference will be given to those whose interests complement ongoing research and who will take advantage of the University of Missouri Research Reactor (MURR). A Ph.D. chemist with an international reputation and exceptional research and teaching capabilities is sought. Candidates should submit a curriculum vitae, a brief description of research plans, and arrange to have three letters of recommendation sent from people familiar with their capabilities in chemistry to: **Professor Silvia Jurisson, Radiochemistry Faculty Search Chair, 123 Chemistry Building, University of Missouri, Columbia, MO 65211-0002.** The selection process will begin on January 7, 1996 and continue until the position is filled. *Applications from women and minority candidates are particularly encouraged. MU is an Equal Opportunity/Affirmative Action Employer.*

Invertebrate Zoologist. The Department of Biology at Illinois Wesleyan University has a tenure-track opening at the **ASSISTANT PROFESSOR** level in invertebrate zoology. The candidate should have a Ph.D., a broad background in biology, and a strong commitment to undergraduate excellence. Responsibilities include teaching invertebrate zoology (introductory course and upper-level seminar), the invertebrate lectures of the general biology course (six lectures), two general biology labs each semester, and a general education course. Teaching load is 24 contact hours per year. Facilities are housed in a new $25 million science center. Appointment begins August 1996. Applicants should submit a letter of application, curriculum vitae, transcripts, a one page statement of teaching philosophy, and three letters of recommendation (including comments on teaching effectiveness) to: **Dr. Bruce B. Criley, Chair, Biology Department, Illinois Wesleyan University, P.O. Box 2900, Bloomington, IL 61702-2900.** Applications will be considered beginning November 1, 1995 and continue until the position is filled. *An Equal Opportunity Employer; women and minorities are encouraged to apply.*

ECOLOGY

The Department of Biological Sciences at Rutgers University in Newark invites applications for a **TENURE-TRACK** faculty position in Ecology. Outstanding applicants in all fields of specialization are encouraged to apply, but this research-oriented department is particularly interested in candidates working in physiological, community, or landscape ecology who would bring to the department strong technological, quantitative, or theoretical skills. The successful candidate is expected to develop and maintain an active research program. Teaching on the graduate and undergraduate levels is expected. Please send résumé and three letters of recommendation by December 15, 1995, to: **Dr. G. Miller Jonakait, Professor and Chairman, Department of Biological Sciences, 101 Warren Street, Rutgers University, Newark, NJ 07102.** *Rutgers University is an Equal Opportunity Employer.*

PLANT ECOLOGY

The Department of Biology, Division of Ecology and Organismal Biology at The University of Memphis (formerly Memphis State University) invites applications for a tenure-track **ASSISTANT PROFESSOR** position in plant ecology. Applicants must have a Ph.D.; postdoctoral experience is preferred. Successful candidate will develop a strong, extramurally funded research program, direct M.S. and Ph.D. students, participate in teaching General Ecology, and develop upper-division and graduate courses in area of expertise. Preference will be given to applicants whose research interfaces/complements existing research interests.

Applicants should submit a letter of application, a curriculum vitae, a concise description of research and teaching interests, three reprints, and names of three references to: **Dr. Harold R. Bancroft, Department of Biology, Division of Ecology and Organismal Biology, The University of Memphis, Memphis, TN 38152. Telephone: 901-678-2592; Email: BancroftHR@MSUVX1.Memphis.edu; FAX: 901-678-4746.** Evaluation of applications will begin January 16, 1996 and continue until the position is filled. *Women and minority candidates are encouraged to apply. The University of Memphis is an Affirmative Action/Equal Opportunity Employer.*

ECOLOGIST–The Department of Biology at New Mexico State University expects to fill a tenure-track **ASSISTANT PROFESSOR** position in ecology for fall 1996. We seek an ecologist working with large-scale systems (ecosystem-level, landscape, or regional) with focus on the importance of animals (preferably vertebrate) for those systems. We also welcome applications from ecologists with a research focus on large-scale patterns of animal diversity and/or the conservation implications of those patterns. The successful candidate will develop a vigorous research program and will teach at the graduate and undergraduate levels, including participation in introductory biology courses. Ph.D. required by date of hire; postdoctoral experience preferred. The Department has a flourishing program in ecology and evolution, and offers research opportunities in the Jornada Long-Term Ecological Research program and other Southwestern environments. Submit curriculum vitae, concise statements of research and teaching interests, and the names, addresses, and telephone numbers of at least three references to: **Dr. Laura F. Huenneke, Ecology Search Committee, Department of Biology, New Mexico State University, Las Cruces, NM 88003.** Deadline for applications: 30 November 1995. *New Mexico State University is an Equal Employment Opportunity/Affirmative Action Employer. Offer of employment contingent upon verification of individual's eligibility for employment in the United States.*

ECOLOGY, BEHAVIOR

The University of North Carolina at Chapel Hill invites applications for a tenure-track position at the level of **ASSISTANT PROFESSOR** in the Department of Biology. We seek an outstanding individual in ecology or in animal behavior. We particularly encourage applications from individuals who use innovative methods to address integrative questions in these fields. The appointment will be effective July 1, 1996. Please submit a curriculum vitae, a statement of research and teaching interests, and three letters of recommendation to: **Ecology and Behavior Search Committee, Department of Biology, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-3280.** Priority will be given to applications received by December 1, 1995. *The University of North Carolina is an Equal Opportunity/Affirmative Action Employer and strongly encourages applications from women and minorities.*

Full-time **ASSISTANT PROFESSOR**; tenure system; Michigan State University, beginning August 16, 1996. 75% in Lyman Briggs School, a residential undergraduate program in the College of Natural Science, and 25% in a suitable life-science department. Ph.D. in organismal, cell, or molecular biology required with strong interest in undergraduate teaching essential. Evidence of innovative teaching techniques favorably considered. Published research and the guidance of student research projects expected. Salary commensurate with experience ($35,000 to $40,000). Letter of application accompanied by a curriculum vitae and names of three references should be sent by December 1, 1995 to: **Edward Ingraham, Director, Lyman Briggs School, E-27 Holmes Hall, Michigan State University, East Lansing, MI 48825-1107.** *Members of protected classes are especially encouraged to apply.*

VISITING ASSISTANT PROFESSOR IN PHARMACOLOGY
UNIVERSITY OF HOUSTON

An immediate opening exists for a Visiting **ASSISTANT PROFESSOR** (non-tenure) in the Department of Pharmacological and Pharmaceutical Sciences at the University of Houston. Duties of the position include study of the function, signal transduction, and regulation of prejunctional adrenoceptors using neuronal cell culture and whole organ techniques; supervision of graduate students; grant preparation; and development of novel research initiatives. The successful candidate will have a Ph.D. in pharmacology or related field, at least three years of productive postdoctoral experience, and excellent communication skills. Experience in cell culture and cellular/molecular pharmacology is preferred. Salary will be commensurate with qualifications. Applicants should send a curriculum vitae and the names of three references to: **Dr. Douglas C. Eikenburg, Chair, Department of Pharmacological and Pharmaceutical Sciences, University of Houston, Houston, TX 77204-5515.** Review of applications will begin November 10, 1995 and continue until the position is filled. *The University of Houston is an Equal Opportunity/Affirmative Action Employer; minorities, women, veterans, and persons with disabilities are encouraged to apply.*

BEHAVIORAL NEUROSCIENCE

ASSISTANT PROFESSOR. The Department of Psychology at Yale University expects to make an appointment at the rank of Assistant Professor in behavioral neuroscience effective July 1, 1996. The Department emphasizes research interests in the analysis of learning and memory; however, outstanding candidates in all subspecialties including cognitive neuroscience are encouraged to apply. Applicants are expected to provide high-quality teaching at the undergraduate level and in a graduate Behavioral Neuroscience program, and to have exhibited (or shown very clear promise of) excellence in research. Applicants should send a letter of application, a curriculum vitae, papers or reprints, and should arrange for three letters of recommendation to be sent to: **Chair, Behavioral Neuroscience Search Committee, Department of Psychology, Yale University, P. O. Box 208205, New Haven, CT 06520-8205.** Deadline for completed applications: February 15, 1996. *Yale is an Equal Opportunity/Affirmative Action Employer, and applications from women and minority group members are especially encouraged.*

ECOSYSTEM/LANDSCAPE ECOLOGY

The Biology Department of George Mason University is seeking an **ASSISTANT PROFESSOR** for a tenure-track position to begin 1 September 1996. Applicants with research interests in the management of wetland or terrestrial ecosystems, and focusing on the dynamics interactions of plants, microorganisms, soils, and/or hydrology, are preferred. The successful candidate will be expected to develop an externally funded research program and contribute to both the undergraduate program in Biology and the graduate programs in Environmental Science and Public Policy, including supervision of M.S. and Ph.D. Students. Teaching responsibilities will likely consist of courses in the applicant's specialty and in the core curriculum. To apply, please send curriculum vitae, letter of appreciation, and three letters of recommendation, to be received no later than December 1, 1995, to: **Chairman, Ecologist Search Committee, 3E1, Department of Biology, George Mason University, Fairfax, VA 22030-4444.** *Affirmative Action/Equal Employment Opportunity.*

BIOLOGY

ASSISTANT PROFESSOR. Plant biologists who employ molecular, genetic, developmental, ecological, or evolutionary tools and perspectives in their research are encouraged to apply. The successful candidate will hold the Ph.D. degree in Plant Biology or Botany and will be expected to teach two undergraduate courses per semester and develop a research program that can involve undergraduates. Send curriculum vitae, letter of application addressing teaching and research philosophies as they interface with undergraduates, three letters of recommendation, and a mini-description of two or three courses for junior and senior biology majors that you would like to offer, to: **Dr. Derek Barkalow, Chair, Biology Department, Stetson University, DeLand, FL 32720.** Deadline for receipt of these materials is November 24, 1995. Start-up date is August 16, 1996.

Scripps Institution of Oceanography
Center for Clouds, Chemistry, and Climate

Scripps Institution of Oceanography (SIO) at the University of California, San Diego (UCSD) invites applications for appointment to a research position in the area of atmospheric chemistry.

The research appointments parallel University of California faculty appointments, but are without teaching requirements. For this atmospheric chemistry position applications for the appointment at assistant, associate, or full research scientist will be considered. Salary is commensurate with experience and based on the University of California salary scale. Partial salary support will be provided from various sources subject to availability of funds.

Candidates must hold a Ph.D and have demonstrated their ability to conduct high quality research. Research area may include chemistry of tropospheric games and aerosols, with an emphasis on atmospheric observations. The successful candidate will be expected to establish an extramurally funded research program and to involve SIO graduate students. There will be opportunities to interact with an international and interdisciplinary group of researchers in clouds and climate,. and to participate in on-going field experiments involving aircraft, ships, and surface observations.

Closing date is January 31, 1996. Applicants should send a curriculum vitae, selected reprints, a list of at least three references and a brief statement of research interests to:

V. Ramanathan, Director
Center for Clouds, Chemistry, and Climate
Scripps Institution of Oceanography
University of California, San Diego
9500 Gilman Drive
Mail Code 0239
La Jolla, CA 92093-0239

The University of California is an equal opportunity employer.

BIOLOGICAL PROCESS SCIENCES

Investigator/Senior Investigator

SmithKline Beecham, a world leader in pharmaceutical research, currently has an opportunity for an Investigator to join our Biological Process Sciences department.

The successful candidate will evaluate technology for viral screening assays, track and report progress on project development, qualify assay protocols, prepare SOPs, supervise 1-2 technicians and perform retrospective validation of test article storage conditions. Significant hands-on laboratory work is required.

Qualifications include a PhD in Virology or related field plus at least 1 year postdoctoral experience, or MS with 8 years' relevant experience, or BS with 10 years' relevant experience. Background in viral screening assays to include plaque, co-cultivation and immuno assays and assay by detection of CPE and/or hemadsorption/hemagglutination on indicator lines also required. Willingness to work in BioSafety Level 2 environment and PC skills are imperative. Familiarity with GLP, cGMP and 21 CFR is preferred.

Located in our state-of-the-art research facility in suburban Philadelphia, SmithKline Beecham offers a competitive compensation/benefits package, and a stimulating work environment. Interested applicants should forward their resume to: SmithKline Beecham Pharmaceuticals, Job Code H0280, P.O. Box 2646, Bala Cynwyd, PA 19004. We are an Equal Opportunity Employer, M/F/D/V.

SmithKline Beecham
Pharmaceuticals
Challenging the natural limits.

Wayne State University

THE DEPARTMENT OF PHARMACOLOGY, WAYNE STATE UNIVERSITY SCHOOL OF MEDICINE, invites applications for **TENURE TRACK POSITIONS** at **The ASSISTANT/ASSOCIATE PROFESSOR** level.

Candidates using contemporary approaches to study signal transduction, vesicular trafficking, cell cycle regulation, transcriptional regulation, and protease/protease inhibitors are preferred. Responsibilities include establishing an outstanding independent and extramurally-funded research program and teaching of pharmacology to medical and graduate students. Faculty may participate in the training of graduate students in pharmacology as well as in interdisciplinary degree programs in cancer biology and/or molecular toxicology.

Opportunities are available for research interactions with faculty in the Karmanos Cancer Institute, the Institute for Chemical Toxicology, the Center for Molecular Medicine, the Neuroscience Program and the Neurotrauma Center.

Candidates should have a Ph.D. or equivalent degree with appropriate postdoctoral training. Highly competitive salary and start-up packages are available for these positions. Applications will be accepted until the positions are filled. A CV, a statement of research interests and reprints of up to three relevant publications should be forwarded to: **Dr. David Kessel, Chair, Faculty Search Committee, Wayne State University, School of Medicine, Department of Pharmacology, 540 E. Canfield, Room 6374, Detroit, MI 48201.** Candidates should arrange to have three letters of reference sent directly to Dr. Kessel.

Wayne State University is an equal opportunity/affirmative action employer. All buildings, structures and vehicles at WSU are smoke-free. Wayne State University - People working together to provide quality service.

Purdue University

Solid-Earth Geochemist

The Department of Earth and Atmospheric Sciences, Purdue University, invites applications for a tenure-track position at the Assistant Professor level. We seek an outstanding Ph.D. solid-earth geochemist with a strong background in earth materials who will develop an innovative research program. Areas of specialty might include, but are not restricted to crust mantle processes, mineral physics, reaction kinetics, environmental geochemistry, and biogeochemistry. Preference will be given to candidates interested in pursuing research at the interface of special study areas of the department and university. The successful applicant is expected to have the ability to obtain external support for his/her research program, as well as the ability to teach courses in mineralogy and petrology and introductory and advanced courses in their area of expertise.

The Department of Earth and Atmospheric Sciences consists of 28 full-time faculty, and confers B.S., M.S., and Ph.D. degrees in both solid-earth and atmospheric sciences. First-rate facilities are available within the department, and elsewhere on the campus, for performing essentially all wet/dry geochemical investigations. Additional information on the department is available on Internet's World Wide Web at http://meteor.atms.purdue.edu.

Applicants should submit a resume, transcripts, and a statement of research and teaching interests and objectives, and the names, addresses, and telephone/fax numbers of at least three references to:

Geochemist Search Committee
Department of Earth & Atmospheric Sciences
1397 Civil Engineering Bldg.
Purdue University
West Lafayette, IN 47907-1397

Deadline for receipt of applications is January 1, 1996.

Purdue University is an equal opportunity/affirmative action employer and encourages applications from women and members of minority groups.

Myriad Genetics, located adjacent to the University of Utah, is a biotechnology company focused on the isolation of significant disease-causing genes. We currently have immediate openings for:

Scientists
Requires a PhD or equivalent and a strong background in molecular biology, informatics or genomic research.

Molecular Biology Technicians
Requires an M.S. degree or B.S. degree and a minimum of two years molecular biology experience. ABI sequencing and/or linkage analysis experience preferred.

We offer a competitive compensation package and a highly stimulating, interactive environment. Please send or fax your C.V., including references to: Barbara Berry, Human Resources Dept. SM, Myriad Genetics, Inc., 390 Wakara Way, Salt Lake City, UT 84108. Fax: 801-584-3640. EOE.

MYRIAD

PLANT PHYSIOLOGIST

The Department of Biology at the University of Minnesota-Duluth invites applications for a tenure-track **ASSISTANT PROFESSOR** position in plant physiology beginning September 1, 1996. The position requires a Ph.D. (by 9/1/96) and prior teaching experience; postdoctoral research experience is desirable. Responsibilities will include: teaching plant physiology, general biology, and advanced specialty topics; actively participating in graduate education (emphasis at M.S. level); establishing a strong, externally funded research program in plant physiology; and advising students. Research focus is open and collaborative efforts will be encouraged. Model systems may include vascular plants, algae, fungi, or bryophytes. The successful applicant should have demonstrated competence with molecular approaches. Send curriculum vitae, copies of undergraduate and graduate transcripts, a statement of teaching/research interests and goals, up to three reprints, and three letters of recommendation that address teaching and research potential to: **Dr. Randall E. Hicks, Search Chair, Department of Biology, 10 University Drive, University of Minnesota, Duluth, MN 55812.** Applications must be postmarked by December 1, 1995. Only completed applications can be considered. *The University of Minnesota is an Equal Opportunity Educator and Employer.*

FACULTY POSITION
UNIVERSITY OF PENNSYLVANIA

The Microbiology Department in the School of Dental Medicine is seeking an outstanding scientist for a tenure-track faculty position at the **ASSISTANT/ASSOCIATE PROFESSOR** level. The successful candidate is expected to develop a high-quality independent research program in basic viral-host cell mechanisms funded by extramural support. Preference will be given to an established investigator with an ongoing research program. Appointment at the Associate Professor level requires a strong record of research productivity, funding, and excellence in teaching. The candidate is expected to teach modern aspects of microbial infectious disease to dental students, establish a vigorous research program, interact with faculty at Pennsylvania, and be involved in graduate student training. The candidate must have either a Ph.D./D.M.D. or M.D. and extensive postdoctoral training. A curriculum vitae, three letters of recommendation, a statement of research interests, and pertinent publications should be sent to: **Dr. Gary H. Cohen, Professor and Chair, Department of Microbiology, School of Dental Medicine, University of Pennsylvania, 4010 Locust Street, Philadelphia, PA 19104.** *University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer. Females and minorities are encouraged to apply.*

ASSISTANT PROFESSOR
Molecular/Developmental Biology

The University of Rochester anticipates an opening for an **ASSISTANT PROFESSOR** of Biology. Applicants must have demonstrated outstanding potential for independent work in the area of Molecular or Developmental Biology. The Biology department consists of 22 faculty who employ genetic and molecular approaches to problems in cell, molecular, and developmental biology; evolutionary biology; and ecology. Additional faculty with allied interests are located in the adjacent Medical School. Applicants should submit before December 1, 1995, a curriculum vitae, bibliography, description of research and teaching interests, and should request three references to send letters of recommendation to: **Chair, Molecular/Developmental Search Committee, Department of Biology, University of Rochester, Rochester, NY 14627.**

The University of Rochester is an Affirmative Action/Equal Opportunity Employer.

Biology–MacMurray College, a private United Methodist liberal arts college, is seeking a full-time faculty member in Biology. **TENURE-TRACK.** Qualifications: Ph.D., teaching experience, and expertise in the following areas: cell biology, genetics, biochemistry, microbiology, and histology. Will also share teaching introductory course. Salary and rank commensurate with education and experience. Please send letter of application, curriculum vitae, teaching philosophy, graduate transcripts, and the names and telephone numbers of three references to: **Dr. Jim Goulding, Vice President for Academic Affairs, MacMurray College, 447 East College, Jacksonville, IL 62650.** Deadline is November 17, 1995. *Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

McGill

FACULTY POSITION
SYSTEMS NEUROPHYSIOLOGY/PAIN
MCGILL UNIVERSITY

Applications are invited for a full-time **TENURE-TRACK** position to be held jointly between the Department of Physiology, Faculty of Medicine, and the Faculty of Dentistry. The successful applicant will be a system neurophysiologist with established expertise in pain research who will be expected to establish a successful research program and to contribute to the development of a multidisciplinary research initiative. Responsibilities will also include teaching medical and dental students. Rank and salary will be commensurate with experience. Applications must be received by December 15, 1995. The appointment will commence as soon as a suitable candidate is found. Please forward a curriculum vitae and the names of three referees to:

Dr. P. B. Noble
Department of Oral Biology
Faculty of Dentistry
McGill University
3640 University Street
Montreal, QC H3A 2B2 Canada

In accordance with Canadian immigration requirements, this advertisement is directed to Canadian citizens and permanent residents. McGill University is committed to equity in employment.

Biology. Clarkson University is seeking applications for a tenure-track position at the **ASSISTANT PROFESSOR** level. Postdoctoral experience is required and research should be in the area of Biochemistry/Protein Chemistry using molecular approaches. Research interactions with the Senior Scientists at the W. Alton Jones Cell Science Center in Lake Placid would be encouraged. Anticipated teaching load is two courses a semester, and may include: Biochemistry with Lab, Biotechnology, Molecular Biology, and some duties in the Introductory sequence. Clarkson University is located in a college-town environment in the foothills of the Adirondack Mountains. The Department offers degrees at the Bachelor's, Master's and Doctoral levels (M.S. and Ph.D. in conjunction with the Department of Chemistry). Send curriculum vitae, statement of research and teaching interests, and the names of three references to: **Dr. Michael H. Roberts, Department of Biology, Box 5805, Clarkson University, Potsdam, NY 13699-5805.** Applications will be reviewed on arrival; however, applications arriving by November 17, 1995 will receive full consideration. *Clarkson University is an Equal Opportunity/Affirmative Action Employer.* Position No. 24.

American University In Cairo (AUC). Applications are invited for one faculty opening at the **ASSISTANT, ASSOCIATE,** or **FULL PROFESSOR** level for a biologist to teach, in English, General Biology and a section of the core curriculum's required course in Scientific Thinking. Preference will be given to specialists in environmental biology and/or botany. Ph.D. required. AUC particularly seeks applicants with a demonstrated record or strong promise of excellence in teaching. Scholarly/research experience or potential is expected. Normal teaching load is nine hours per semester. Two-year appointment (renewable) begins September 1996. Rank, salary according to qualifications and experience. For expatriates, housing, roundtrip air travel, plus schooling for up to two children included. Write with curriculum vitae to: **Dr. Andrew Kerek, Provost, The American University in Cairo, 866 United Nations Plaza, Suite S-517, New York, NY 10017,** preferably before November 30, 1995. *AUC is an Equal Opportunity Employer.*

FACULTY POSITION

The Department of Surgery, Wayne State University, invites M.D./Ph.D. applicants to manage a Burn Research Laboratory presently interested in translocation of bacteria and other infectious problems of burned patients. Applicants must have an emphasis in immunology/microbiology and have postdoctoral experience. Please send curriculum vitae to: **J. K. Prasad, M.D., Director, Burn Center, 3V-26, 4201 Saint Antoine, Detroit, MI 48201.** *Wayne State University is an Affirmative Action/Equal Opportunity Employer.*

POSITIONS OPEN

ANIMAL PHYSIOLOGIST

The Trinity College Department of Biology invites applications for a tenure-track position at the level of **ASSISTANT PROFESSOR** to begin in the fall term, 1996. Applicants must have a Ph.D. (postdoctoral experience is preferred, but not required) and should have a record of teaching and scholarship in neurobiology so that participation in our Neuroscience Program is possible. Annual teaching duties include an animal physiology course (preferably comparative animal physiology) and participation in team-taught courses of introductory biology and of neurobiology; in addition, it is expected that an advanced course in the area of the applicant's interest and a course for non-majors will be offered in alternate years. The candidate should pursue a program of research in the neurosciences in which upper-level students may participate. Excellent facilities and start-up funds are available. Send curriculum vitae (including statements on approach to teaching, and of current research interests), graduate and undergraduate transcripts, and have three letters of recommendation forwarded to: **Robert H. Brewer, Chair, Department of Biology, Trinity College, Hartford, CT 06106.** Evaluation of applications will begin 1 December 1995 and continue until the position is filled. *Trinity College is an Equal Opportunity/Affirmative Action Employer and actively seeks applications from minorities and women.*

FACULTY POSITIONS IN PHYSIOLOGY, IMMUNOLOGY, NEUROBIOLOGY AND BIOCHEMISTRY

The Department of Biology at Georgia State University invites applications for three anticipated **TENURE-TRACK** positions, one each in physiology, immunology, and neurobiology. Areas of research interest for the physiology position should complement existing research strength in renal physiology and neuroendocrinology. The area for the immunology position should involve molecular approaches. The area for the neurobiology position should be in computational neuroscience. Appointments will start on or before September 15, 1996. Applicants will be considered at the advanced assistant professor or more senior level. It is desirable for applicants to have current funded research programs. Successful candidates will be expected to maintain independent research programs, supervise M.S. and Ph.D. students, and participate in instruction at the undergraduate and graduate levels. The Department has several core facilities housing numerous shared equipment, including: DNA/protein synthesizers and sequencers, a cell sorter, a phosphoimager, capillary electrophoresis and chromatography systems, image analysis and electron microscopes, and data analysis work stations. Applications including curriculum vitae, reprints, statement of research plans, and three letters of reference should be sent to: **Chair of Search Committee (Physiology, Immunology or Neurobiology), Department of Biology, P.O. Box 4010, Georgia State University, Atlanta, GA 30302-4010.** Deadline for receipt of application is December 11, 1995.

The Department of Chemistry invites applications for an anticipated **TENURE-TRACK** position in Biochemistry. Candidates will be expected to establish a vigorous, externally funded research program and attract students in an expanding Ph.D. program. Expertise in protein or nucleic acid chemistry is preferred; access to departmental NMR facilities (300, 400, 500, 600 MHz) and Biology core facilities is available. Excellence in teaching at all levels expected. Send curriculum vitae, description of proposed research, and three letters of recommendation by December 11, 1995 to: **Prof. Dixon, Department of Chemistry, Georgia State University, Atlanta, GA 30303.**

Georgia State University, a unit of the University System of Georgia, is an Equal Opportunity/Affirmative Action Employer.

DIRECTOR, Cytogenetics Laboratory: The Division of Human Genetics of the Children's Hospital Research Foundation (CHRF) seeks a ABMG Clinical Cytogenetics certified or eligible M.D. or Ph.D. to direct its service and research activities. Responsibilities include amniotic cells, CVS, blood and tumor karyotype and FISH analyses, and development of research program. Academic rank determined by qualifications. Send curriculum vitae, bibliography and three letters of recommendation to: **Gregory A. Grabowski, M.D., Director, Division of Human Genetics, CHRF, 3333 Burnet Avenue, Cincinnati, OH 45229-3039.** *Children's Hospital Medical Center is an Affirmative Action/Equal Opportunity Institution. Women and minorities are encouraged to apply.*

TIGR
THE INSTITUTE FOR GENOMIC RESEARCH

FACULTY POSITIONS

The Institute for Genomic Research is a not-for-profit research institute with interests in structural, functional, and comparative analysis of genomes and gene products in viruses, eubacteria, pathogenic bacteria, archaea, and eukaryotes, both plant and animal, including humans (see Fleischmann, et al, *Science*, 269: 449-604, 1995, Lee, et al, *PNAS*, 92: 8303-8307, 1995, Adams, et al, *Nature*, 377 Supp: 3-174, 1995, and Fraser, et al, *Science*, this issue). The Institute has recently moved to its new campus in Rockville, MD, in the greater Washington, D.C. metropolitan area, and is close to the National Institutes of Health, Johns Hopkins University, The University of Maryland, and other research institutes and biotechnology companies. The new facility has over 60,000 sq. ft. of laboratory and office space and TIGR plans to expand its research faculty in the following areas:

MICROBIOLOGY.

Applicants are sought with a strong microbiology, molecular biology and/or molecular evolutionary background to lead a research effort in the discovery of new species of archaea and in the comparative characterization of their genomes, metabolism, and physiology. We are also seeking applicants to lead a research program concerned with the discovery and characterization of new species of marine microorganisms utilizing our research vessel SEA TIGR, a converted 63 ft. North Sea trawler.

CELL BIOLOGY.

Whole genome sequencing of prokaryotes, archaea, and expressed sequence tag (EST) analysis of higher eukaryotes has revealed a large number of new genes with no counterparts in the nucleic acid or protein databases. We are interested in applicants with strong backgrounds in cell/molecular biology to characterize these new gene products, to examine their mode of expression and their structure, and to determine their role in cell metabolism and physiology.

Scientists who have a Ph.D., M.D., or equivalent degree with at least two years postdoctoral experience are encouraged to apply by submitting a cover letter, a *curriculum vitae*, including a bibliography, a statement of research interests, and the names of three references to:

Human Resources Department, The Institute for Genomic Research, 9712 Medical Center Drive, Rockville, MD 20850, Tel: (301) 838-0200, FAX: (301) 838-0208, e-mail: facpost@tigr.org

TIGR is an equal opportunity employer and encourages qualified women and minority candidates to apply

OPPORTUNITIES IN MOLECULAR DIAGNOSTICS

SmithKline Beecham, a worldwide leader in pharmaceuticals research, currently has excellent opportunities for several scientists to join our newly organized Molecular Diagnostics Department. All candidates must have a comprehensive understanding of their discipline and relevant literature based on several years of post- graduate experience.

Investigator

Responsibilities include: the development of high density arrays of clones from libraries; performing hybridization of these arrays with various probes for eliminating redundant genes and/or identifying differentially expressed genes; and validating the approaches and transferring the technology to other scientists in the department. Qualifying candidates will have a Ph.D., plus demonstrated expertise in library screening, gene cloning and characterization, and preferably experience in the use of subtractive cloning methods, genomics and sequencing. Refer to Job Code H0433.

Scientist/Senior Scientist

Using robotics, this individual will be responsible for synthesizing oligonucleotides, automated sequencing of DNA and assisting in arraying clones. Results will be presented at departmental, team and divisional meetings, reflecting accuracy and adherence to SOPs. Candiates must have an B.S./M.S. plus specialist technology/skills, and the ability to apply new techniques and adapt them to relevant research, and be able to apply own research efforts to Program/Feasibility Study goals. Refer to Job Code H0435.

Scientist/Senior Scientist

The successful candidate will independently perform experiments in advanced genetics aimed at constructing and analyzing cDNA libraries, perform subtraction techniques to construct libraries enriched for messages specific to tissues or disease states, develop and evaluate methods for differentially labeling probes in analysis of these cDNA libraries, and evaluate these libraries using high density arrays of clones. Candidates must have a B.S./M.S. plus specialist technology/skills, be able to apply new techniques and adapt them to relevant research, and be able to apply own research efforts to Program/Feasibility Study goals. Refer to Job Code H0434.

Located in our state-of-the-art research facility in suburban Philadelphia, SmithKline Beecham offers an excellent compensation/benefits package and a stimulating work environment. Qualified candidates should send CV/resume and salary requirements, indicating desired Job Code to: SmithKline Beecham Pharmaceuticals, Job Code _____, P.O. Box 2646, Bala Cynwyd, PA 19004. We are an Equal Opportunity Employer, M/F/D/V.

SB
SmithKline Beecham
Pharmaceuticals
Challenging the natural limits.

Research Associate (Job #1078) - Grain Traits. The successful candidate will interact in a team environment focusing on plant intermediary metabolism. Skills in a variety of disciplines are required, including "hands-on" experience in recombinant DNA techniques (e.g., gene isolation, vector construction, PCR) and protein isolation and purification methods. Knowledge/ experience in plant metabolism and physiology is a plus. The individual should be highly motivated, able to work with relatively little supervision, be efficient at problem solving, and be able to communicate effectively. Requirements: BS or MS in molecular biology, biochemistry or related field with a minimum of 3 years relevant bench experience.

Research Associate (Job #1080) - Grain Traits. The successful candidate will join a team involved in optimizing gene expression in transgenic plants. Applicants must have significant skills in nucleic acid analysis (including DNA/RNA purification, sequencing, cloning, PCR, Southerns and Northerns) and experience with computer analysis programs. Good organizational skills and the ability to interact effectively in a broad multidisciplinary environment will be important. Requirements: BS with 4 years of directly related experience, or an MS with 2 years of experience.

Pioneer offers an excellent salary/benefits program, career growth potential, excellent facilities and a cooperative research environment. Send CV and cover letter indicating Job # by November 10 to: **Cindy Letizia, Grain Traits, Pioneer-Hi-Bred International, Inc., 7300 NW 62nd Ave. - Box 1004, Johnston, IA 50131-1004.**

PIONEER.

Wayne State University

DEVELOPMENTAL BIOLOGY / EVOLUTION

The Department of Biological Sciences invites applications for several tenure-track positions at the full, associate and assistant professor level. We are seeking faculty members with their primary research interest in molecular genetic analysis of development and evolution. We are also interested in individuals using molecular genetic analysis of development to address evolutionary questions. **The department is undergoing a significant expansion and will consider applicants in related areas.**

Substantial space and start-up funds will be made available to the successful candidates. The department occupies a new seven-story research building with animal rooms, greenhouses, microscopy facilities, Drosophila support facilities, and a molecular biology core facility.

Applicants must have demonstrated excellence in research and the potential for the highest quality teaching. Candidates will be expected to establish and maintain an extramurally funded research program and participate in both undergraduate and graduate teaching programs.

Women and minority candidates are encouraged to apply. Review of candidates will begin immediately and continue until all positions are filled.

Send curriculum vitae, a description of current and long-range research plans, three representative papers and the names of four references to:

Jack Lilien
Chair, Department of Biological Sciences
Wayne State University
Detroit, MI 48202

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Wayne State University - People working together to provide quality service
All buildings, structures and vehicles at WSU are smoke-free.

TWO TENURE-TRACK ASSISTANT PROFESSOR POSITIONS

UNIVERSITY OF NEBRASKA-LINCOLN

ANIMAL PHYSIOLOGICAL ECOLOGIST- Applicants should possess research expertise in investigating important evolutionary or ecological processes at the physiological level that address responses to variable and changing environments. Research programs should include a significant field component. Candidates will be expected to develop a vigorous research program and assume teaching responsibilities at the undergraduate and graduate levels, preferably including an introductory Human Physiology course.

PLANT MOLECULAR BIOLOGIST- Candidate's expertise should involve the use of molecular and cellular approaches to solve fundamental problems in plant biology. Candidates will be expected to develop a vigorous research program and assume teaching responsibilities in both undergraduate and graduate level courses in plant molecular biology and general plant biology.

Scientists with a Ph.D. and postdoctoral experience are encouraged to apply for either position by sending a curriculum vitae, statement of research interest and arrange for three letters of reference to be sent by December 1 to: **Chairperson (Specify specific position), School of Biological Sciences, 348 Manter Hall, University of Nebraska, Lincoln, NE 68588-0118.** Positions will be open until a suitable candidate is selected. *UNL is committed to a pluralistic campus community through AA/EO and is responsive to the needs of dual career couples. We assure reasonable accommodation under ADA; contact Dr. T. Jack Morris for assistance.*

VANDERBILT UNIVERSITY SCHOOL OF MEDICINE

FACULTY POSITIONS IN POPULATION GENETICS AND GENETIC EPIDEMIOLOGY

Vanderbilt University School of Medicine seeks applicants to fill new tenure track faculty positions as part of a multidisciplinary program expansion genetics. Established scientists as well as new investigators having Ph.D. or M.D. degree(s) are invited to apply. Areas of emphasis include human population genetics, genetic epidemiology and study of polygenic disorders. Expertise in quantitative methods such as mapping of disease susceptibility loci and large scale automated genotyping as well as development of original methods and research designs are desired. Successful applicants will receive attractive start up packages and have opportunities for a variety of interdepartmental collaborations involving basic and clinical sciences. Salary and faculty rank will be commensurate with qualifications and experience. Vanderbilt University School of Medicine is an equal opportunity/affirmative action employer.

Candidate should send a short statement of research interest, a CV and name of three reference familiar with their work to:

Genetics Initiative
c/o Dr. D.K. Granner
Vanderbilt University
School of Medicine
707 Light Hall
Nashville, TN 37232-0615

DIAGNOCURE INC.

Located in the heart of Québec City, DiagnoCure is a young rapidly growing medical company developing for cancer diagnosis and treatment. DiagnoCure will employ up to 25 scientists and technologists and has immediate openings for experienced to lead its research unit in the following fields:

Immunodiagnostics: Biotechnology/pharmaceutical experience in development of diagnostics kits. Experience with labeling of monoclonals and fluorescence-based assays would be an advantage.

Recombinant Vaccines: Experience in recombinant vaccine techniques with different vectors, familiarity with anti-idiotypic approaches and synthetic peptides.

Recombinant Peptides: Experience in screening of peptide libraries and reagent development.

Molecular Biology: Experience in characterization of proteins, cDNA cloning and protein structure-function studies.

Each head scientist will report to the Vice President R & D and will play a key role with its scientific advisory committee in determining research strategies. The head scientists will be responsible for the implementation and management of the research programs with the help of support staff. The candidates must have the skills to lead and motivate the staff to perform effectively in a multidisciplinary team under tight schedules.

We offer a highly competitive salary, commensurate with experience, along with the advantages of a progressive, entreprenurial environment.

Please send your c.v. to the:

Vice President of R&D
DiagnoCure Inc.
70, rue Dalhousie, bur. 110
Québec (Québec)
G1K 4B2 Canada

Committed to quality, diversity and equal employment opportunities.

Career Opportunities at St. Jude Children's Research Hospital

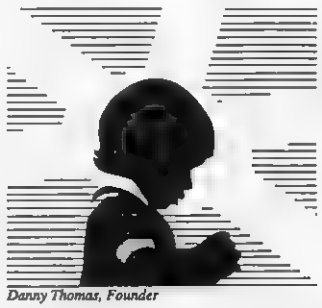
St. Jude Children's Research Hospital, a premier research center for biomedical research, offers postdoctoral fellowship opportunities available in the basic and clinical sciences. Recent M.D., Ph.D., D.V.M., or Pharm.D. fellows are eligible to train with leading scientists in several basic biomedical research laboratories, including biochemistry, experimental oncology, genetics, experimental hematology, immunology, molecular pharmacology, tumor cell biology and virology/molecular biology. Additional opportunities are also available in the clinical sciences, including infectious diseases, hematology/oncology, pharmaceutical sciences, pathology and laboratory medicine, radiation oncology and diagnostic imaging. An interdisciplinary effort in cell and gene therapy has been initiated. SJCRH currently has 155 postdoctoral fellows funded from peer-reviewed grants, outside fellowships and institutional sources. Ten named fellowships are currently available.

Fellowship awards are given for up to three years and are based on merit, recommendation and the promise of a productive career in biomedical research. Stipends are highly competitive.

Applicants should provide a curriculum vitae, including a brief description of their research interests, and have three references. These should be forwarded to:

Academic Programs
St. Jude Children's Research Hospital
332 North Lauderdale
Memphis, TN 38105

SJCRH is an Affirmative Action/ Equal Opportunity Employer



Danny Thomas, Founder

Research Scientist

VICAL INCORPORATED, a leader in the development of gene-based pharmaceutical products for human gene therapy is currently seeking a Research Scientist. The incumbent will be responsible for development and validation of biological assays, including the development of *in vitro* and *in vivo* models for the evaluation of transfection and expression and the determination of specific activity of plasmid DNA-based pharmaceuticals. The ideal candidate will possess the following:

- Extensive background in biological assay development and validation, FACS analysis, histological staining techniques, in situ hybridization, and immunoassay development
- Extensive background in cell and molecular biology
- Ph.D. in life sciences with post doctoral and industrial experience in biological assay development

Please send your resume to **Vical, Inc. Human Resources, 9373 Towne Centre Drive, Suite 100, San Diego, CA 92121 or fax to (619) 453-3452.**

EOE

Faculty Positions in Neurobiology

University of Maryland School of Medicine
Department of Anatomy

The Department of Anatomy is undergoing major expansion and rebuilding. New faculty are being recruited in neurobiology. Positions are available at all ranks.

The Department is located in a newly opened building. Significant expansion of research resources is underway, including core laboratories in molecular biology, tissue culture, neuroanatomy, imaging, and neurophysiology.

Successful candidates will have a Ph.D. or equivalent, outstanding ability in teaching, and exceptional achievement in their field of research, including the potential to attract external funding. Investigators in the Department use approaches ranging from molecular biology to behavior but the common focus is understanding the *organization*, *function* and *development* of neural networks. We particularly encourage applicants investigating the:

- interplay of gene expression and neural activity in neural network development.
- neurobiological bases of learning and memory.
- development and functional organization of chemosensory systems.

The Department teaches a 9.5 week course - "Structure & Development" - in the medical school curriculum. New faculty are expected to participate 3-5 weeks in this team taught course. Graduate teaching is also encouraged.

For best consideration, applications should be received before February 1, 1996. Candidates should send a C.V., summary of research accomplishments and goals, and the names of 3-5 individuals from whom recommendations may be obtained to:

Dr. David V. Smith
Chair, Faculty Search Committee
Department of Anatomy
University of Maryland School of Medicine
655 West Baltimore Street
Baltimore, MD 21201-1559

The Department is eager to diversify its faculty. We encourage minorities and women to apply. The University of Maryland is an AA/EEO/ADA employer.

University of Miami

University of Miami

Rosenstiel School of Marine and Atmospheric Science

TRITIUM LABORATORY

is seeking a
Research Assistant/Associate/Professor

With interest in Oceanography or Groundwater Hydrology

Ph.D. in math or a natural science required.

The Tritium Laboratory does low-level tritium measurements in oceanographic and hydrological projects in cooperation with US and foreign institutions. Funding for the operations and staff of about five, is derived from semi-commercial measurement service to institution and businesses, and from regular research grants and contracts from US agencies.

The applicant should have research interests in Hydrology or Oceanography, a strong background in measurement techniques and basic knowledge in chemistry. Previous experience in low-level beta counting is very desirable, and an undergraduate degree in nuclear engineering would be a plus. We envision that the scientist would pursue their own fundable research interests, improve and modernize the present measurement system, and oversee routine laboratory operations.

Send resume and three references to:

Search Committee Chair, TL
UM/RSMAS/MAC
4600 Rickenbacker Cswy.
Miami, FL 33149

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Faculty Positions in Genetics

Dartmouth Medical School

Through the generous support of the Lucille P. Markey Charitable Trust, Dartmouth Medical School is recruiting faculty in genetics. Appointees are expected to maintain excellent research programs that address fundamental questions in an area of genetics that includes microbial genetics, cancer genetics, gene therapy, mouse or human genetics, or model systems such as yeast, drosophila, and zebra fish. Appointees will also participate in the teaching of genetics to graduate and medical students.

Applications are especially encouraged from established investigators at the Associate Professor level, but candidates of all ranks are encouraged to apply. Appointments will be in an appropriate basic science department at Dartmouth Medical School.

Review of applications will begin on November 1, 1995. Please send a CV, a description of research interests, and arrange for three letters of recommendation to be sent to: Professor Constance Brinckerhoff, Ph.D.; Dartmouth Medical School; Box 7200, Vail Building; Hanover, NH 03755.

An equal opportunity/affirmative action employer.

Director

Program in Human and Medical Genetics

The University of Colorado Health Sciences Center, in Denver, seeks candidates for the position of Director of a newly formed program in Human and Medical Genetics. Entities involved in this program include the Schools of Medicine, Dentistry, Nursing and Pharmacy, the Eleanor Roosevelt Institute for Cancer Research, and The Childrens Hospital. The Program Director is expected to integrate already substantial efforts in clinical genetics and genetics research and education on the campus, to develop new research initiatives, and to develop and direct a doctoral program in human and/or medical genetics. The successful candidate should be a senior M.D., M.D./Ph.D. investigator with a national or international reputation in basic and/or clinical genetic research. Administrative experience, and the ability to lead and interact effectively with substantial resources to this position, including competitive personal compensation and start up funds for the Director, faculty positions, research space, and funds for program initiatives. Review of applicants will begin on Jan. 15, 1996, and continue until a successful candidate is identified. Letters of interest, together with c.v., should be sent to:

Stephen I. Goodman, M.D., Head
Genetics Search Committee, Box B-161
University of Colorado Health Sciences Center
4200 East Ninth Avenue
Denver, Colorado 80262

The University of Colorado Health Sciences Center is committed to equal opportunity and affirmative action.

INTERNATIONAL GRADUATE STUDENT FELLOWSHIP PROGRAM at the AMERICAN MUSEUM OF NATURAL HISTORY CENTER FOR BIODIVERSITY AND CONSERVATION

Program: **The International Graduate Student Fellowships Program** at the Museum's Center for Biodiversity and Conservation provides an outstanding opportunity for non U.S. citizens to study a diversified curriculum in systematics, biodiversity, conservation, and public policy. Students are able to choose among the numerous offerings of four major universities to create a graduate program from which they will bring an interdisciplinary mix of skills and experience to bear on the environmental problems of their countries.

Students are part of a joint Museum-university program offering the Ph.D. degree. Under the direction of a Museum curator, students will attend classes and seminars at both the Museum and their chosen university.

Eligibility: This program is open to non U.S. citizens. Applications are particularly encouraged from students from developing nations. Applicants must have a bachelors degree, and be able to fulfill university admission requirements. These include TOEFL and Graduate Record Examinations.

Awards: The fellowship will provide travel assistance, stipend support for 12 months, and tuition. Support is for 4 years, renewable annually providing the student remains in good standing.

Contact: Applicants should first contact the Office of Grant and Fellowships to discuss their interests, background and eligibility for the Program.

Application Procedure: Students must simultaneously apply to the Museum AND to one of 4 cooperating universities depending on field of study.

1. Application to the Museum is on prescribed forms and will include a resume of the student's academic background, work experience, research interests, statement of purpose, and the names of two references familiar with the student's work. Application deadline is January 1, 1996.

2. Application to one of the universities listed below should be made based on field of interest and submitted by the university's deadline date. Students should contact the university for application forms.

Evolutionary Biology: Office of Admissions, The Graduate School, The City University of New York, 33 West 42 Street, New York, NY 10036-8099 Deadline: April 15, 1996

Molecular Biology/Biological Sciences: Office of Student Affairs, The Graduate School of Arts and Sciences, Columbia University, 107 Low Library, New York, NY 10027 Deadline: January 4, 1996

Biology/Systematics: Department of Biology, Graduate Program, Yale University, P.O. Box 208103, New Haven, CT 06620-8103 Deadline: January 2, 1996

Entomology: Office of Admissions, The Graduate School, Cornell University, Sage Graduate Center, Ithaca, NY 14853-6201 Deadline: January 10, 1996

Request Museum application forms and further information from:

Office of Grants and Fellowships
American Museum of Natural History
Central Park West at 79th Street
New York, NY 10024
Telephone: 212-769-5467 Fax: 212-769-5495
E-mail: bynum@amnh.org

POSITIONS OPEN

UNIVERSITY OF NORTH CAROLINA AT CHARLOTTE

The Biology Department at University of North Carolina (UNC) Charlotte seeks to fill the following vacancies. (1) **BIOLOGY CHAIR:** 12-month appointment, beginning July 1996. Department offers B.A., B.S., M.A., and M.S. degrees, and is developing a proposal for an interdisciplinary Ph.D. program. Faculty teach and conduct research in a range of traditional fields and participate in multidisciplinary programs such as biotechnology and medical technology, and conduct collaborative education in association with hospitals and research centers. Candidates must have earned doctorate, research and grant experience, and provide strong leadership in research and undergraduate/graduate education. Previous administrative experience preferred. Appointment will be at Associate Professor or Professor, with salary and teaching load competitive. Teaching and research areas are open, but the Department has declared an emphasis in biomedical/biotechnology and seeks to strengthen those areas. Candidates should send a letter outlining professional accomplishments relevant to this position, a curriculum vitae, and names of three references to: **Dr. Farid Tranjan, Chair, Biology Search Committee, UNC Charlotte, Charlotte, NC 28223.** (2) **PHYSIOLOGIST: ASSISTANT/ASSOCIATE PROFESSOR,** tenure-track. Ph.D. and postdoctoral experience required. Candidates must have an independent research program, a record of securing extramural funding, a commitment to quality undergraduate/graduate teaching, and ability to teach mammalian physiology. Candidates should send a letter outlining qualifications, a curriculum vitae, and names of three references to: **Chair, Physiology Search Committee, Biology Department, UNC Charlotte, Charlotte, NC 28223.**

PROFESSOR AND CHAIR OF PHARMACOLOGY AND CELL BIOPHYSICS

The University of Cincinnati College of Medicine invites applications and nominations for **CHAIR** of this department, which has a history of academic and research excellence. Current faculty support includes two training grants and two NIH Merit awards. The College receives approximately $60 million in sponsored program awards and has significant interdisciplinary programs of distinction in cardiology, neuroscience, and oncology. Faculty have been internationally recognized for expertise in the generation/evaluation of transgenic mice. The college is developing an interdisciplinary Structural Biology program with 750, 600, and 500 MHz NMRs.

We seek qualified candidates with distinguished records in research, teaching, and service, and demonstrated excellence in leadership and administration. Individuals with nationally and internationally recognized and funded research programs in molecular approaches to pharmacology or related disciplines are particularly encouraged to apply. The Chair is responsible for medical and graduate instruction in pharmacology as well as for maintaining and developing outstanding, externally supported research programs within the department. Review of candidates will begin in late 1995. Send nominations and applications (include letter of interest, curriculum vitae, and three references) to: **Jerry B. Lingrel, Ph.D., Pharmacology Search Committee Chair, University of Cincinnati College of Medicine, 231 Bethesda Avenue, Cincinnati, OH 45267-0524.** *University of Cincinnati is an Affirmative Action/Equal Opportunity Employer. Women, Minorities, Disabled, Veterans encouraged to apply.*

CHAIR
Department of Biology and Chemistry

The University of Montevallo invites applications for an anticipated position as **CHAIR** of the Biology-Chemistry department. Ph.D. in either biology or chemistry required; prefer zoologist capable of teaching comparative vertebrate anatomy, or chemist with background in inorganic or physical chemistry. Teaching experience and a strong dedication to undergraduate teaching and research are required. Administrative experience is desirable. Send curriculum vitae and three letters of recommendation to: **Dr. Steve O'Donnell, Acting Chair, Department of Biology and Chemistry, The University of Montevallo, Montevallo, AL 35115.** Review of applications begins immediately and continues until position is filled. *The University of Montevallo is an Affirmative Action/Equal Opportunity/Americans with Disabilities Act Employer.*

ASSOCIATE DIRECTOR
Applied Marine Research Laboratory (AMRL)

The AMRL, a self-supporting research program affiliated with Old Dominion University (ODU), has a position available immediately for applicants with strong experience and interest in providing a leadership role in marine and environmental research. Responsibilities include operational management, supervision of managers for several laboratories, proposal and report preparation and review, research administration, and representation of the program to funding agencies and the public. This position also serves as a principal investigator on multidisciplinary projects for government agencies and the private sector. A Ph.D. in marine or related environmental science, and at least five years of experience in designing and conducting research projects, is required. Preference will be given to an applicant with evidence of significant extramural support and experience in leading multidisciplinary teams of investigators. While the area of expertise is open, special consideration will be given to applicants with experience in environmental toxicology or pollution ecology. The successful applicant should also have good communication and personnel management skills, as well as a hands-on approach to working with technical staff and academic investigators. Adjunct faculty appointments are possible with various academic departments. Some travel is required. An application letter outlining professional experience and interests; a curriculum vitae; and names, addresses, and telephone numbers of at least four professional references must be sent to: **ODURF, P.O. Box 6369, Norfolk, VA 23508-0369.** Expected hire date is February 1, 1996. *Equal Employment Opportunity/Affirmative Action Employer.*

ASSOCIATE PROFESSOR CHEMICAL EDUCATION

As part of the ongoing commitment to improve chemical education at the University of Nebraska-Lincoln, the Department of Chemistry invites applications for a **TENURE-TRACK POSITION** in Chemical Education. The candidate should have a Ph.D. degree in Chemistry or a closely related science; have substantial experience in chemical education, especially innovative general chemistry teaching; and should have established a nationally competitive, funded program in chemical education research. Experience in outreach activities, laboratory development, computer-aided instruction, and multimedia based education are also desirable. Please submit a curriculum vitae, a summary of research plans and a teaching portfolio, and arrange to have three letters of recommendation sent to: **Professor Gerard Harbison, Search Committee Chair, Department of Chemistry, University of Nebraska-Lincoln, Lincoln, NE 68588-0304. Telephone: 402-472-9346; FAX: 402-472-2044; Email: gerry@wendigo.unl.edu.** Deadline is November 30, 1995 or until position is filled.

The University of Nebraska-Lincoln is committed to a pluralistic campus community through Affirmative Action and Equal Opportunity and is responsive to the needs of dual career couples. We assure reasonable accommodation under the Americans with Disabilities Act; please contact **Diane Stevens, Telephone: 402-472-2745; FAX: 402-472-9402** *for assistance.*

ASSISTANT PROFESSOR OF GENETICS

Applicants are sought for a full-time, **TENURE-TRACK** position in an area of Genetics. Applicants will be expected to establish a competitive research program using genetic analysis on a model system. Suitable areas of research include, but are not limited to, genome structure and function, genetics of development, neurogenesis, signal transduction, and cellular processes. Teaching obligations will include undergraduate courses in genetics or cell biology and upper-division and graduate teaching in the applicant's area of expertise. A Ph.D. and relevant postdoctoral experience is required. Applicants should send curriculum vitae, statement of research interests, up to five reprints, and the names and addresses of three referees to: **Genetics Search Committee, Department of Biological Sciences, Simon Fraser University, Burnaby, BC V5A 1S6 Canada.** Applications should be received by December 15, 1995. All positions are subject to budgetary authorization.

In accordance with Canadian Immigration requirements, this ad is directed in the first instance to Canadian citizens and permanent residents of Canada.

Simon Fraser University is committed to the principle of equity in employment and offers Equal Employment Opportunities to qualified applicants.

Animal Ecologist. Tenure-track, **ASSISTANT PROFESSOR,** beginning fall 1996 in the Department of Biology at Colorado College: a private, undergraduate, highly-selective, liberal arts institution enrolling 1900 students. Responsibilities include teaching introductory ecology, advanced ecology, and vertebrate zoology and development of a field-oriented research program involving undergraduates. Ability to teach population genetics highly desirable. Ph.D. required. *The College particularly seeks candidates who understand the perspectives of women and minorities in the curriculum and invites candidates to identify their strengths or experiences in these areas.* Applicants should send: 1) cover letter describing professional background, goals, strengths, and experiences, 2) statement of teaching philosophy, 3) description of current and future research plans, 4) curriculum vitae, 5) official transcripts, and 6) four letters of recommendation to: **Richard Storey, Chair, Department of Biology, Colorado College, Colorado Springs, CO 80903** by December 8, 1995. *Colorado College is an Equal Opportunity Employer and a participant in the minority faculty registry.*

ASSISTANT PROFESSOR OF MOLECULAR MICROBIOLOGY

The Department of Biology at Virginia Tech invites applications for a tenure-track, **ASSISTANT PROFESSOR** position for August 1996. Applicants should have postdoctoral experience and a broad background in microbiology. The successful candidate will develop a competitive research program using molecular techniques to explore fundamental processes in microbial cells. Responsibilities will include undergraduate and graduate teaching in microbiology. A letter of application, curriculum vitae, statement of both research and teaching goals, and three letters of reference should be received by January 5, 1996. Correspondence should be sent to: **Allan A. Yousten, Department of Biology, Virginia Tech, Blacksburg, VA 24061-0406.** *Virginia Tech has a strong commitment to increasing the diversity of the faculty and seeks a broad spectrum of candidates including women, minorities, and people with disabilities.* Persons with disabilities desiring accommodations in the application process should notify **Dr. Yousten** by application deadline.

HUMAN GENETICS AT MICHIGAN

The Department of Human Genetics is seeking a **TENURE-TRACK FACULTY** member in Human/Mammalian Population Genetics and/or Quantitative Genetics. We are searching for a creative investigator who will establish an independent and productive research program. He or she will join an established department of 24 members whose research interests include molecular genetics, developmental genetics, and population genetics of both rare and common human diseases. Attractive salary compensation, generous start-up funds, and excellent research facilities are available. Please send résumé, statement of research interests, and names of three references to: **Thomas D. Gelehrter, M.D., Professor and Chair, Department of Human Genetics, M4708 MS II/0618, University of Michigan Medical School, Ann Arbor, MI 48109-0618.** *The University of Michigan is an Equal Opportunity/Affirmative Action Employer, and specifically invites and encourages minority and women applicants.*

Plant Biologist: Canisius College has a tenure-track opening for an **ASSISTANT PROFESSOR.** The ideal candidate will contribute to both our pre-professional and environmental biology curricula by offering electives such as plant biology, medicinal botany, and cell biology, and will teach in our introductory biology sequence. The development of a research program that involves undergraduates is expected. Research in plant-animal interactions would have broad appeal to our students. Send curriculum vitae, research plan, teaching philosophy, transcripts, and the names of three references to: **Dr. Paula Dehn, Chair Biology, Canisius College, Buffalo, NY 14208** by November 15, 1995. For additional information **Email: Dehn@wehle.Canisius.edu; Telephone: 716-888-2555.**

Biochemist: Susquehanna University invites applications for a tenure-track appointment at the **ASSISTANT PROFESSOR** level, effective September 1996. Ph.D. in biochemistry, a strong background in chemistry. Ability to teach effectively and conduct a research program with undergraduate students. Program accredited by ACS. Submit résumé, statement of research interest, transcripts, and three letters of reference by December 15, 1995: **Dr. Christopher Janzen, Department of Chemistry, Susquehanna University, Selinsgrove, PA 17870.** *Susquehanna is an Equal Opportunity/Affirmative Action Employer. Women and minority candidates are encouraged to apply.*

POSTDOCTORAL POSITION

CELL CYCLE REGULATION OF CELL MEMBRANE SYNTHESIS

Investigation of modification of the rate controlling enzyme in the pathway of phospholipid formation by regulatory kinases using contemporary techniques of molecular and cell biology. Study of phospholipase activation/signalling by growth factors and its role in cell cycle regulation of membrane synthesis and trafficking (J. Biol. Chem. 269:3858, 1994). Position available immediately and recent PhD required. Send curriculum vitae with names of three references to: **Dr. Suzane Jackowski, Department of Biochemistry, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105.** *An Affirmative Action/Equal Opportunity Employer.*

MERCATOR GENETICS

TRANSLATING KNOWLEDGE INTO THERAPIES

Mercator Genetics was capitalized in the Spring of 1993 for the purpose of applying new genomic technologies to the identification of genes that cause major human diseases. The discovery of disease genes will enable the development of improved diagnostic tests which guide the use of increasingly specific therapeutic agents.

Scientists

Positions are available for scientists with a Ph D. and Post doctoral experience in human molecular genetics, molecular biology, cell biology, or related research. Desired candidates should have expertise in all areas of genetic and positional cloning including bioinformatics, genetic mapping, cDNA library construction and screening, high-throughput genotyping, large scale automated sequencing and YAC construction.

Bioinformatics

You will lead the effort to develop the algorithms and software systems necessary to support positional cloning including sequencing, sequence analysis, gene finding, database and laboratory management systems. You will also be responsible for the supervision of company-wide systems management. Position requires a MS or Ph.D. in the sciences and an expertise in UNIX based systems with demonstrated programming skills. Experience in both computer science and biology preferred.

In addition to an exciting and challenging research environment, Mercator offers competitive salaries and benefits, an employee stock option plan and an attractive San Francisco Bay Area location. For consideration, please send resume with cover letter to: Mercator Genetics, Inc. Human Resources, 4040 Campbell Avenue, Menlo Park CA 94025. Fax: (415) 617-0883, E-Mail (ASCII only, please): personnel@mercator.com. *Equal Opportunity Employer*

EASTERN MICHIGAN UNIVERSITY

Department of Biology

Three tenure-track Assistant Professorships available Fall 1996. The department has 22 faculty with graduate program concentrations in Ecology and Organismal Biology, Molecular and Cellular Biology, Physiology, and General Biology. Successful applicants will be expected to develop a research program capable of attracting external funding, and participate in graduate programs and an emerging new undergraduate curriculum. Positions require a Ph.D., teaching experience and productive research.

Eukaryotic Molecular Geneticist (Position F9608), whose research addresses evolutionary questions. Applicants must be capable of teaching molecular genetics, introductory genetics and evolution

Immunologist (Position F9609), capable of teaching immunology at the introductory and graduate levels, and possibly in the areas of microbiology, molecular biology, and/or virology.

Vertebrate Physiologist (Position F9610), capable of teaching introductory level courses in human anatomy and physiology, and advanced courses in his/her specialty.

Submit letter of interest, curriculum vitae, statement of teaching interests and philosophy, description of research, recent reprints, and three letters of reference by November 17 to the address below. For additional information, contact Dr Douglas Shapiro, Department Head, Phone (313) 487-4242, Fax (313) 487-9235.

Position #(see above)
204 King Hall
Eastern Michigan University
Ypsilanti, MI 48197

Eastern Michigan University is an affirmative action/equal opportunity employer. We strongly encourage women and members of minority groups to consider these opportunities.

Make a Difference in the Quality of Life

Baxter Healthcare Corporation's Biotech Group, Hyland Division, is significantly enhancing its biopharmaceutical development and manufacture of therapeutic proteins with the opening of our new, large-scale production facility in Thousand Oaks, California.

PURIFICATION SUPERVISOR – We seek an experienced Purification Supervisor to assume responsibility for the technical and managerial aspects of the production area as it relates to operation of affinity and ion exchange chromatography columns and support systems functions. The position consists of organizing, monitoring and actually running the Purification Production area.

We require a BS/MS in Biochemistry or Biochemical Engineering, and 4 years related experience and/or training; or equivalent combination of education and experience. Pharmaceutical/biotech experience preferred. Employment is contingent upon the result of a drug screening test.

For immediate consideration, mail/FAX resume with salary history, to: **Baxter Biotech Group, Human Resources Dept., 1700 Rancho Conejo Blvd., Thousand Oaks, CA 91320. FAX: (805) 375-6810.** Equal Opportunity Employer.

Biotech Group • Hyland Division • Thousand Oaks, CA

Baxter

POSITIONS OPEN

DEPARTMENT OF CELL BIOLOGY AND ANATOMY UNIVERSITY OF ARIZONA

Applications are invited for a tenure-eligible faculty position in the Department of Cell Biology and Anatomy. Preference will be given to candidates at the **ASSISTANT PROFESSOR** level, although more senior appointments may be made for exceptional individuals. Outstanding applicants in all contemporary research areas will be considered, especially those that complement the existing strengths of the Department (cell and molecular biology, developmental biology, neurobiology, imaging). Candidates must have a Ph.D. or equivalent degree and postdoctoral experience with demonstrated ability in research. The successful candidate is expected to develop and/or maintain a vigorous, independent, extramurally funded research program and to participate in a team taught Medical Gross Anatomy course, as well as teach graduate students. To ensure consideration, applications should be received by December 1, 1995; however, applications will be accepted until the position is filled. Applicants should submit a curriculum vitae, a detailed description of present and future research goals, and names and addresses of three references to: **Dr. Clark Lantz, Chairperson, Faculty Recruitment, Department of Cell Biology and Anatomy, University of Arizona, Health Sciences Center, 1501 North Campbell Avenue, P.O. Box 245044, Tucson, AZ 85724-5044.** *The University of Arizona is an Equal Opportunity/Affirmative Action/Equal Access Employer. Women and minorities are encouraged to apply.*

TENURE-TRACK FACULTY POSITION University of Missouri-Columbia

The University of Missouri-Columbia (MU) seeks a tenure-track **ASSISTANT, ASSOCIATE,** or **FULL** Professor to be jointly appointed in the colleges of Veterinary Medicine and Agriculture, Food and Natural Resources. The successful candidate will be expected to lead an outstanding independent research program in an area of cell or structural biology of importance to basic biomedical aspects of veterinary medicine and agriculture, teach electron microscopy at the graduate level, and direct (at 20% effort) a recently established, campus-wide Electron Microscopy Core Facility under the auspices of the MU Molecular Biology Program. Experience in both plant and animal systems is preferred, but not required; postdoctoral experience is necessary. Applicants should submit, by January 31, 1996, a description of research plans, curriculum vitae, selected reprints, and names, Email addresses, and telephone/FAX numbers of three professional references to: **Dr. Margaret A. Miller, Co-chair, Cell Ultrastructure Faculty Search Committee, UMC Veterinary Medical Diagnostic Laboratory, P. O. Box 6023, Columbia, MO 65205. Telephone: 314-882-6811; FAX: 314-884-7544; Email: miller@vmdl.missouri.edu.** *The University of Missouri-Columbia is an Affirmative Action/Equal Opportunity/American Disabilities Act Institution. Women and members of minority groups are especially encouraged to apply.*

FACULTY POSITION IN BACTERIAL PATHOGENESIS

The Department of Microbiology at the College of Physicians and Surgeons of Columbia University is soliciting applications for a tenure-track faculty position at the level of **ASSISTANT PROFESSOR.** Applicants should have a strong background in prokaryotic genetics and experience in an area related to the interaction of pathogens with host cells. Applicants should have demonstrated a high level of academic achievement and the potential for developing an active research program. The Department has an active graduate program, supported by a training grant, and all faculty are expected to maintain a strong commitment to graduate education. Substantial start-up resources are available. Interested individuals should send a curriculum vitae and a statement of research interests and should also arrange to have three letters of reference sent to: **The Microbiology Search Committee, Department of Microbiology, College of Physicians and Surgeons, Columbia University, 701 West 168th Street, New York, NY 10032. Email: horan@cumbib.bmb.columbia.edu.**

Columbia University is an Affirmative Action/Equal Opportunity Employer.

FACULTY POSITION Microbiology/Microbial Pathogenesis

The Department of Microbiology of The University of Texas Health Science Center at San Antonio invites applications for several **TENURE-TRACK** faculty positions that will be filled at the junior to senior levels. The department is especially interested in recruiting individuals whose research program will focus on aspects of microbial pathogenesis, host-pathogen interactions and inflammation. However, outstanding applicants in all areas of microbial molecular biology are encouraged to apply. Applicants should mail curriculum vitae, a statement of current and future research goals, and arrange to send three letters of reference by January 24, 1996 to: **Dr. Joel B. Baseman, Professor and Chair, Department of Microbiology, The University of Texas Health Science Center at San Antonio, 7703 Floyd Curl Drive, San Antonio, TX 78284-7758.** *The University of Texas Health Science Center at San Antonio is an Equal Employment Opportunity/Affirmative Action Employer.*

YORK UNIVERSITY

Department of Psychology in the Faculty of Arts invites applications for a tenure-track position at the **ASSISTANT PROFESSOR** level in Perception. Of particular interest are candidates with expertise in the area of the psychophysics of spatial vision, motion perception or colour vision and the applications of basic research to human performance. Duties include teaching in both the undergraduate and graduate programs. Candidates should have a promising publication record and program of research. Enquiries and applications, with curriculum vitae, three letters of reference, and relevant reprints, should be directed to: **Prof. Sandra Pyke, Chair, Department of Psychology, Faculty of Arts, York University, North York, Ontario M3J 1P3, Canada.** Deadline for applications is December 15, 1995. *York University is implementing a policy of employment equity, including Affirmative Action for women faculty. In accordance with Canadian immigration requirements, priority will be given to Canadian citizens and permanent residents of Canada.* All positions at York University are subject to budget approval.

UNIVERSITY OF CALIFORNIA LOS ANGELES INORGANIC FACULTY POSITION

The Department of Chemistry and Biochemistry of the University of California, Los Angeles intends to make a tenure-track faculty appointment in inorganic chemistry at the **ASSISTANT PROFESSOR** level. Applicants with interests in any area of inorganic chemistry will be considered. Candidates must give evidence of potential distinction in scholarship and teaching. Applications should include a curriculum vitae, a description of proposed research not exceeding four pages, and letters of recommendation from at least three professional references. Applications should be completed no later than December 15, 1995 and directed to: **Chair, Inorganic Search Committee, Department of Chemistry and Biochemistry, University of California, Los Angeles, CA 90095-1569. FAX: 310-206-9130.** *The University of California is an Equal Opportunity Employer and encourages applications from women and minority candidates.*

MICROBIOLOGY POSITION—The University of Mississippi Department of Biology seeks an **ASSISTANT PROFESSOR** (nine-month, tenure-track) beginning fall 1996. A Ph.D. with emphasis in microbiology and publications in peer-reviewed journals is required. University-level teaching experience desirable. Duties include teaching undergraduate courses and graduate courses in the area of specialty. Peer-reviewed publications and the development of an extramurally funded research program involving graduate students is expected. Candidates with research experience in physiological, ecological, molecular, or immunological aspects of disease are preferred. Send curriculum vitae, letter of teaching and research interests, names, telephone numbers, and addresses of four references to: **Dr. Al Mikell, Chair, Microbiology Search Committee, Department of Biology, University of Mississippi, University, MS 38677. Telephone: 601-232-7204 Before 31 December 1995.** *The University of Mississippi is an Affirmative Action/American with Disabilities Act/Equal Opportunity Employer.*

LECTURER/ACADEMIC COORDINATOR POSITION IN CHEMISTRY

Applications are invited for the position of **LECTURER/ACADEMIC COORDINATOR** in the Department of Chemistry beginning in the spring of 1996, pending budgetary approval. Duties of the position are the experimental reorganization and supervision of the freshman chemistry laboratory, recitation and training of graduate student instructors. A doctoral degree in chemistry and previous teaching experience in chemistry are prerequisites. The nine-month salary is $28,600 to $36,000, depending on experience, with the possibility of additional income for summer school teaching. The appointment is for one year with possible reappointment for three additional one-year terms, depending on good performance. Apply to: **Chair, Lecturer Recruiting (No. 65), Department of Chemistry, University of California, Berkeley, CA 94720-1460.** The deadline for receipt of applications is December 15, 1995. *The University of California is an Equal Opportunity/Affirmative Action Employer.*

ASSISTANT PROFESSORSHIP Animal Developmental Biologist

The Waksman Institute at Rutgers, The State University of New Jersey, is inviting applications for a **TENURE-TRACK POSITION.** The position is a joint appointment in the Department of Microbiology and Genetics and requires teaching at the graduate and undergraduate level. Applicants should submit a curriculum vitae, list of publications, three letters of reference, and a summary of research plans to: **Dr. Ruth Steward, Search Committee Chairperson, Waksman Institute, Rutgers, The State University of New Jersey, Box 759, Piscataway, NJ 08855-0759.** *Rutgers University is an Affirmative Action/Equal Opportunity Employer.*

NEW HEALTH EVALUATIONS SCIENCES DEPARTMENT

The University of Virginia School of Medicine is establishing a new health evaluation sciences department devoted to the discovery and development of new approaches to and uses of patient description, prognosis, clinical, and genetic risk assessment, information transfer, medical practice and research strategies. The department's efforts will enable clinicians, administrators, and others to more precisely evaluate the efficacy of existing medical practice and more accurately test new approaches.

We are now seeking talented and innovative leaders in the fields of biostatistics, clinical trial design, clinical epidemiology, health services research, outcomes evaluation, medical decision-making, and clinical informatics to help create an integrated clinical research and development department. All efforts will be closely aligned with clinical and basic science research and educational initiatives at UVA.

The department has widespread support within the School of Medicine, Medical Center and University. A number of full-time, **TENURE-TRACK** positions are available for clinicians and investigators at all academic levels. Newly renovated space, equipment, research associates, and secretarial support are provided. Individuals with academic or industrial expertise are invited to send cover letter and curriculum vitae to:

William A. Knaus, M.D., Evelyn Troup Hobson Professor and Chair, University of Virginia School of Medicine, Box 600, Charlottesville, VA 22908.

The University of Virginia is an Equal Opportunity/Affirmative Action Employer.

ANIMAL PHYSIOLOGIST

Tenure-track **ASSISTANT PROFESSOR** of Biology begin fall 1996. Ability to stimulate student interest, direct undergraduate research, interact with diverse student population. Master's degree required, Ph.D. preferred. Teaching responsibilities include vertebrate physiology, cell and molecular biology, advanced courses in areas of the candidate's interest. Research interest should include application of molecular techniques to organismal biology. Application deadline December 15, 1995. Send letter of interest, résumé, brief statement of teaching and research interests, and telephone numbers or Email addresses of three references to: **Dr. Norval Kneten, Dean, Science and Humanities, Texas Wesleyan University, 1201 Wesleyan Street, Fort Worth, TX 76105-1536.** *Affirmative Action/Equal Opportunity Employer.*

SYMPOSIUM

Stem Cells, Lineage, and Plasticity of the Differentiated State

Sponsored by
Cutaneous Biology Research Center
Massachusetts General Hospital/Harvard

9am - 5pm

Friday May 17,1996

Armed Services YMCA (adjacent to Building 149)
Charlestown Navy Yard
Charlestown MA

Scheduled Speakers:

Nicole M. Le Douarin

Embryogenesis:Stem Cells, Lineage and Plasticity
Institut d' Embryologie Cellulaire Ctr Moleculaire du CRNS Nogent-sur-Marne Cedex, France

Elizabeth J. Robertson

Zygotes, Embryos and Embryonal Carcinomas.
Department of Cellular and Developmental Biology
Harvard University

Irving L. Weissman

Development of the Hematolymphoid System from Stem Cells
Department of Pathology & Developmental Biology
Stanford University School of Medicine

Katia Georgopoulos

Development of the Immune System: Stem Cells and Lineages
Cutaneous Biology Research Center
Massachusetts General Hospital/Harvard

Jeremy Brockes

Amphibian Limb Regeneration: Plasticity or Stem Cells?
Ludwig Cancer Research Institute, London, England

Frank E. Stockdale

Muscle Development: Plasticity or Stem Cells?
Department of Medicine
Stanford University Medical Center

Elaine V. Fuchs

Development of the Epidermis: Stem cells or fixed lineages?
Department Molecular Genetics and Cell Biology
University of Chicago

Andrew P. McMahon

Development of the Nervous System: Stem cells, Lineage or Plasticity?
Department of Cellular and Developmental Biology
Harvard University

Open to the Public. Additional information to follow.

POSITIONS OPEN

STATISTICAL/POPULATION GENETICIST

The Medical University of South Carolina (MUSC) offers a tenure-track position as **ASSISTANT** or **ASSOCIATE PROFESSOR** with co-appointment in the Department of Medicine (Division of Endocrinology, Diabetes, and Medical Genetics) and the Department of Biometry and Epidemiology. The ideal candidate will have a Ph.D. or M.D. degree and an established research program in statistical or population genetics related to human disease with or without an involvement in molecular genetics. He or she would maintain an independent research program and participate in ongoing activities at MUSC, notably the genetic epidemiology of diseases afflicting African-Americans. The candidate will be invited to participate in the development of a world-class family registry for positional cloning of diabetes and obesity genes, in linkage analyses and study design, and in genetic epidemiology components complementing other large population-based studies. Send curriculum vitae, statement of research interests, and references to either co-chair of the search committee: **Dr. Tim Garvey, Director, Division of Endocrinology, Diabetes, and Medical Genetics, Telephone: 803-792-2529; FAX: 803-792-4114; Email: tim_garvey@musc.edu,** or **Dr. David Hoel, Chair, Department of Biometry and Epidemiology, Telephone: 803-792-2262; FAX: 803-792-1123; Email: david_hoel@musc.edu, MUSC, 171 Ashley Avenue, Charleston, SC 29425.** *Equal Opportunity/Affirmative Action Employer.*

MICROBIOLOGY LABORATORY COORDINATOR

Microbiology Laboratory **COORDINATOR** sought for the Department of Biology at Texas A&M University. Applicants should have a Ph.D. in Microbiology or related discipline, experience in modern laboratory techniques, and a commitment to undergraduate education. The position will involve lecturing in introductory microbiology and directing undergraduate microbiology laboratories. Opportunities will exist for extensive interaction with both faculty and students, for designing new laboratory exercises, for participation in course development, and for establishing contacts with potential employers of students graduating with B.S. degrees in Microbiology. The Department also supports active research and seminar programs in various areas of microbiology. The salary will be competitive and commensurate with experience. Please send a curriculum vitae and three letters of reference by December 1, 1995, to: **Craig Nessler, Department of Biology, Texas A&M University, College Station, TX 77843-3258.** *Texas A&M is an Equal Opportunity Employer.*

ACOS/EDUCATION—Albuquerque VA Medical Center (VAMC) and University of New Mexico School of Medicine. Responsible for development, coordination, and evaluation of educational programs for health professionals, physicians, trainees, employess, and patients. Minimum requirements: M.D. or Ph.D., directly related experience, eligibility for **FACULTY** appointment at Associate/Professor level in appropriate Department; good communication skills. VA experience desirable. Although applications will be considered until position is filled, selection process will begin on December 15, 1995. Position subject to random drug testing per Federal law. Send cover letter, curriculum vitae, and names of three references to: **Judith A. Fabian, M.D., Chief, Anesthesiology Service, VAMC (112F), 2100 Ridgecrest Drive, SE, Albuquerque, NM 87108.** *Affirmative Action/Equal Opportunity Employer.*

DIRECTOR TRANSGENIC MOUSE CORE FACILITY

The University of Chicago is seeking a **RESEARCH SCIENTIST** to direct a transgenic mouse core facility used by a diverse group of investigators. Appropriate background and training required. Experience with ES cell technology preferable. Send curriculum vitae with names and addresses of three references to:

Kenneth S. Polonsky, M.D.
The University of Chicago
MC 1027
5841 South Maryland Avenue
Chicago, IL 60637

Tenure-track position at the **ASSISTANT PROFESSOR** level, available July 1, 1996, as part of a Departmental growth program. Applicants should have a Ph.D. and/or M.D., relevant postdoctoral experience, a strong research background, and be using state-of-the-art methods to define molecular mechanisms that regulate cell function. Outstanding individuals who will expand and complement current Departmental teaching and research programs in the areas of cardiovascular pharmacology, receptor pharmacology, neuropharmacology, signal transduction, and regulation of cell growth will be considered. Review of applications will begin October 27, 1995. Applicants should send a curriculum vitae, along with a summary of research interests and goals, and arrange to have at least three letters of reference sent to: **Faculty Search Committee, Department of Pharmacology, University of Wisconsin Medical School, 1300 University Avenue, Madison, WI 53706-1532.** *The University of Wisconsin is an Equal Opportunity/Affirmative Action Employer.*

BIOLOGY FACULTY LAKE FOREST COLLEGE

A continuing appointment as **ASSISTANT PROFESSOR** is anticipated (subject to administrative approval) beginning August 1996 for a highly qualified Ph.D. interested in teaching and conducting research with undergraduates in a liberal arts environment. Teaching will include the majors' core course in genetics plus advanced and/or non-major courses to complement research interests. Research plans should engage undergraduates in publishable investigations of current questions in areas such as developmental biology, neurobiology, or immunology. Modern research facilities and start-up funds are available. Postdoctoral experience is preferred. A letter stating teaching and research plans, curriculum vitae, and three letters of reference should be sent by December 1, 1995 to: **Dr. David W. Towle, Chairperson, Department of Biology, Lake Forest College, Lake Forest, Illinois 60045. Email: towle@lfc.edu.** *Applications from minorities and women are actively encouraged.*

VERTEBRATE BIOLOGIST/ECOLOGIST

The Department of Biology of Carroll College, a coeducational, small, Catholic, liberal arts college, invites applications for a full-time (academic year), tenure-track **ASSISTANT PROFESSOR** beginning fall 1996. Ph.D. is required. Teaching responsibilities include Ecology, Comparative Anatomy, Life Science for non-majors, and a portion of a team-taught Introductory Biology course for majors. Candidates should be committed to undergraduate teaching with high academic standards, research and thesis programs and student advising. Aggressive grant seeking and research not required. Send cover letter, curriculum vitae, transcripts, and three letters of recommendation by December 1, 1995 to: **Dr. Bruce Busby, Academic Vice President, Carroll College, 1601 North Benton Avenue, Helena, MT 59625-0002.** *Equal Employment Opportunity.*

UNIVERSITY OF MASSACHUSETTS AMHERST

College of Natural Sciences and Mathematics, Department of Biology. **SENIOR POSTDOCTORAL POSITION** available. An opening is available immediately for an experienced researcher to assume a leading role in a small group studying structure/function relationships of nuclei and chromatin with emphasis on conformational changes that accompany regulatory events. Candidates should have a thorough background and extensive experience in one or more of the following areas: nucleic-acid protein interactions, electron microscopy and image processing, chromatin molecular biology. This position does not provide benefits. Competitive salary commensurate with background and experience. Send curriculum vitae, statement of experience and research goals, and names, addresses, and telephone numbers of three references to: **Dr. Chris Woodcock, Biology Department, University of Massachusetts, Amherst, MA 01003. Telephone: 413-545-2825; FAX: 413-545-1696; Email: chris@bio.umass.edu.** To ensure full consideration, applications should be received by December 1, 1995. Applications will continue to be received after this date and the search will remain open until a successful candidate is identified. *The University of Massachusetts Amherst is an Affirmative Action/Equal Opportunity Employer.*

ASSISTANT PROFESSORSHIP

The Waksman Institute and the Department of Microbiology and Genetics of Rutgers, The State University of New Jersey, invite applications and nominations for a **TENURE-TRACK** faculty position in Protein Chemistry, Protein-Protein Interaction, and Combinatorial Chemistry.

We seek individuals interested in analysis of protein-protein and protein-nucleic acid complexes of biological significance, including recombination, replication, transcription, and translation complexes. We seek individuals with demonstrated ability and commitment to conduct research, to teach at the undergraduate and graduate levels, and to assume other usual academic responsibilities.

The successful candidate will join a multidisciplinary group of investigators in the study of biomolecular structure, function, and design. Salary and rank will be commensurate with experience, background, and professional qualifications.

Applications should include an expression of interest in the position, a current publication list, a curriculum vitae, and confidential letters of recommendation from four professional references. Applications will be accepted until the position is filled. Applications and nominations may be directed to: **Professor Richard H. Ebright, Chairman, Protein Chemistry Search Committee, Waksman Institute, Rutgers, The State University of New Jersey, P.O. Box 759, Piscataway, NJ 08855-0759.**

Rutgers University is an Affirmative Action/Equal Opportunity Employer.

POSTDOCTORAL OPPORTUNITY

An exciting new gene therapy company is seeking an energetic and talented individual to work on signal transduction pathways that are modulated by adenovirus. Candidates should have a Ph.D. in molecular biology or related fields. This position offers competitive salary and benefit package. Interested individuals should send a résumé and the names of three references to:

GenVec, Inc.
Human Resources
Att: JB
12111 Parklawn Drive
Rockville, MD 20852

An Equal Opportunity Employer.

Two **POSTDOCTORAL POSITIONS** available immediately. Position 1) To study opioid receptors and cellular signal transduction mechanisms. Experience in cellular electrophysiology (intracellular recording and/or patch clamp techniques) required. Position 2) To study opioid receptor functions in animal models. Experience in animal behavioral testing and/or psychopharmacology desired. Both positions offer opportunities to incorporate multi-disciplinary approaches to study the actions of opioids. Send curriculum vitae, summary of research interests, and names, addresses and telephone numbers of three references to: **Dr. L. Yu, Department of Medical and Molecular Genetics, Indiana University School of Medicine, Indianapolis, IN 46202. Email: igcm100@indycms.iupui.edu.** *Equal Opportunity Employer.*

POSTDOCTORAL POSITION available to study cell cycle-dependent (and damaged DNA-induced) regulation of eukaryotic DNA replication. A recent Ph.D. degree with experience in molecular biology and/or biochemistry (nucleic acid enzymology in particular) is preferred. Please send a curriculum vitae, research experience, and names of three references to: **Dr. Suk-Hee Lee, Department of Virology and Molecular Biology, St. Jude Children's Research Hospital, 332 North Lauderdale, Memphis, TN 38105. FAX: 901-523-2622.** *Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION

POSTDOCTORAL POSITION is available immediately to study male germ cell genetic modification (*Proc. Natl. Acad. Sci. USA*, 91: 11298; 11303). Experience in molecular biology is required. Send curriculum vitae, names of three references, and a letter describing research skills and interests to: **R. I. Brinster, School of Veterinary Medicine, University of Pennsylvania, 3850 Baltimore Avenue, Philadelphia, PA 19104.**

Shippensburg University

Position Announcement

BIOLOGY, ASSISTANT PROFESSOR. The Department of Biology at Shippensburg University invites applications for a tenure-track position starting August 1996. The successful candidate will be required to have completed an earned doctorate at the time of appointment. Primary teaching responsibilities will include the non-majors' courses Problems of the Environment, Basic Biology, and Introduction to Ecology. The successful candidate will also occasionally teach Ecology and/or Field Zoology for biology majors. A strong commitment to undergraduate teaching is required. Active learning and multimedia instructional skills preferred. Scholarly activity will be required in the area of the candidate's specialty. Applicants should send curriculum vitae, copies of transcripts (both graduate and undergraduate), a statement of teaching philosophy and research interests, plus the names, addresses and telephone numbers of three references to: **Michael R. Marshall, Chair of the Search Committee, Department of Biology, Shippensburg University, 1871 Old Main Drive, Shippensburg, PA 17257-2299.** Receipt deadline for application materials is January 15, 1996. *Shippensburg University is committed to equal employment opportunity. Women, persons of color, veterans, and the disabled are encouraged to apply.*

Postdoctoral Position
Cell & Molecular Biology

A postdoctoral position is available immediately for studies investigating mechanisms of estrogen-dependent transcriptional regulation and growth control in human mammary and ovarian cells. The successful candidate will carry out research to elucidate the function of a novel, estrogen-regulated zinc-finger protein. Experience in molecular biology and cell culture is desirable.

As a US subsidiary of an international Fortune 500 company, Berlex offers an exciting research environment and a competitive two-year fellowship stipend. Our facility is located in the San Francisco Bay Area. For immediate consideration, send your Curriculum Vitae, research summary, and the names, addresses, and telephone numbers of three references, to: BERLEX, HUMAN RESOURCES EMPLOYMENT, 15049 SAN PABLO AVE., Dept. PDF, RICHMOND, CA 94804-0099. EOE.

BERLEX

POSTDOCTORAL FELLOWSHIPS at the WOODS HOLE OCEANOGRAPHIC INSTITUTION

Applied Ocean Physics & Engineering
Biology, Marine Chemistry & Geochemistry
Marine Geology & Geophysics
Physical Oceanography

Applications are invited from new or recent doctorates in the fields of biology, molecular biology, microbiology, chemistry, engineering, geology, geophysics, mathematics, meteorology and physics, as well as oceanography. Recipients of awards are selected on a competitive basis, with primary emphasis placed on research promise.

Fellowships are awarded for 12-18 month appointments with a stipend of $36,000 per year, plus group health insurance and a modest research budget. Recipients are encouraged to pursue their own research interest in association with resident staff. Completed applications must be received by **January 16, 1996** for the 1996-97 awards. Awards will be announced in March. Write for application forms to: **Dean of Graduate Studies, MS #31, P.O. Box S, Woods Hole Oceanographic Institution, Woods Hole, MA 02543-1541. Or call (508) 457-2000, ext. 2200 or Email lcampbell@whoi.edu.**

WOODS HOLE OCEANOGRAPHIC INSTITUTION

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An equal opportunity/affirmative action employer

New York University

Faculty Position
Department of Biology

The NYU Department of Biology is undergoing a program of expansion and development. Twelve faculty have been added in the past 5 years. A senior position is currently available (subject to final budgetary approval), in the area of cellular and molecular physiology. Outstanding candidates in other areas will also be considered. Candidates are expected to have active, externally-funded research programs and to participate in the department's teaching activities at both the undergraduate and graduate levels. The department offers an excellent research environment with a strong molecular focus, substantial start-up packages and modern laboratory facilities. Application letter with curriculum vitae and three letters of reference should be sent to: **Chairman of the Search Committee, Department of Biology, New York University, 1009 Main Building, Washington Square, New York, NY 10003.**

NYU encourages applications from women and members of minority groups.

SCIENTISTS IN DRUG METABOLISM

The Department of Drug Metabolism at Merck Research Laboratories has openings for two highly motivated BS/MS biologists/pharmacologists/animal scientists who are interested in pursuing a career in drug metabolism and supporting the team effort in drug discovery and development.

The positions require a BS/MS or equivalent in biology/pharmacology/animal science (or a related field) with at least three years of research experience. Successful candidates will be responsible for conducting the in-life phase of metabolism studies in rodents and in other laboratory animals (e.g., dog, non-human primates). Candidates should have excellent skills in handling small animals and in performing established surgical procedures (e.g., vein cannulation, bile-duct cannulation) for metabolism or pharmacological studies. The knowledge of handling radiolabeled chemicals and GLP requirements in conducting scientific experiments is desirable but not essential. A working knowledge in drug metabolism is highly desirable.

Merck Research Laboratories are located in Rahway, NJ, approximately 25 miles from New York City. Our salaries, benefits and growth potential are excellent. For consideration, send your resume, with the names of three references to **Merck Research Laboratories, Human Resources, Ad #168, P.O. Box 2000, RY80A-3, Rahway, NJ 07065.** EOE/AA/VH/Employer.

MERCK
Research Laboratories

POSITIONS OPEN

A position as a **POSTDOCTORAL FELLOW** is now available in a children's cancer center. Applicants must have a Ph.D. in a life science and previous experience with the following techniques: cloning and manipulation of DNA fragments into bacterial and/or yeast plasmids; PCR; RT PCR; isolation of DNA; RNA and protein for subsequent analysis with Northern, Southern, and Western blotting; eukaryotic tissue culture and transfection techniques; experience with the use of radioactive isotopes; familiarity with either PC or MAC computer systems. Experience in molecular virology is also desired. Please send curriculum vitae and three reference letters to: **Dr. Judith Margolin, Baylor College of Medicine, 6221 Fannin, MC 3-3320, Houston, TX 77030.** *Baylor College of Medicine is an Equal Opportunity/Affirmative Action/Equal Access Employer.*

POSTDOCTORAL POSITION available immediately to investigate the molecular physiology of normal and disease-causing mutant cystic fibrosis transmembrane conductance regulator (CFTR) chloride channels in intracellular membranes. Studies will utilize digital imaging of single living cells and single-channel patch clamp electrophysiology of native endoplasmic reticulum using novel approaches. Requires Ph.D. or M.D. and electrophysiology, imaging or molecular biology experience. Ability to work independently within group with strengths in ion transport and molecular biology. Send or FAX curriculum vitae, a short statement of research interests and names of three references to: **Dr. J. Kevin Foskett, Director, Ion Transport Core, Institute for Human Gene Therapy and Department of Physiology, University of Pennsylvania School of Medicine, BRB-1, 422 Curie Boulevard, Room 313, Philadelphia, PA 19104-6069. FAX: 215-573-8590.** *The University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer. Women and minorities are encouraged to apply.*

POSITIONS OPEN

RESEARCH FELLOW OR TECHNOLOGIST PREIMPLANTATION GENETICS

This position involves biomedical research and its clinical application in the area of preimplantation genetic diagnosis. The techniques to be used are tissue culture, multicolor-multiprobe-FISH, PCR, RT-PCR, micromanipulation of gametes and embryos, and cytogenetics. A degree in biology and two years of experience in molecular cytogenetics research are preferred. Previous knowledge of embryology not necessary but a plus. The position is located in Livingston, New Jersey (30 minutes from Manhattan). To apply send résumé and two reference letters to: **Santiago Munné, Director of Preimplantation Genetics, The Center for Reproductive Medicine and Science of Saint Barnabas, 101 Old Short Hills Road, Suite 501, West Orange (Livingston), NJ 07052.**

POSTDOCTORAL POSITION
Transcription and Leukemogenesis

An NIH-funded position is available to apply molecular biology and cell culture methods to study the AML1 transcription factor and its leukemic variants in myeloid differentiation and leukemogenesis—see *Mol. Cell. Biol.* 14: 5558, 1994. Send curriculum vitae and names of references to: **Dr. Alan Friedman, Johns Hopkins Oncology Center, Room 3-109, 600 North Wolfe Street, Baltimore, MD 21287. FAX: 410-955-8897.** *Johns Hopkins University is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION

University of California and Veterans Administration Medical Center (VAMC), San Francisco, has a position available immediately for a qualified individual to join a group studying the impact of microgravity and skeletal unloading on bone growth and differentiation. Applicants should have a Ph.D. in the biological sciences, be experienced with cellular and molecular techniques, and be willing and able to work with rodents. Please send curriculum vitae and names of three references to: **Daniel Bikle, M.D., Ph.D., Department of Medicine, VAMC (111N), 4150 Clement St., San Francisco, CA 94121.**

POSITIONS OPEN

POSTDOCTORAL POSITION

A **POSTDOCTORAL POSITION** is available for a recent graduate to investigate the biochemical and genetic mechanisms of radiation-induced apoptosis. Knowledge in one or more of the following techniques a must: DNA, RNA gel electrophoresis, southern and northern blotting, PCR, immunoprecipitation and western blotting. Send or fax curriculum vitae, names of three references, and short statement of research interests to: **Dr. Michael Story, Department of Experimental Radiotherapy-66, U. T. M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, TX 77030.** *An Equal Opportunity/Affirmative Action Employer.* Smoke-free environment.

POSTDOCTORAL FELLOWSHIP available to study the regulation and biosynthesis of alginate, an exopoly-saccharide produced by the phytopathogenic bacterium, *Pseudomonas syringae*. Experience in molecular biology is required; experience in protein purification is also desirable. This project is a collaboration between **Dr. C. Bender**, Oklahoma State University (OSU), and **Dr. A. Chakrabarty**, University of Illinois at Chicago (UIC). Résumés to: **Dr. C. Bender, 110 Noble Research Center, OSU, Stillwater, OK 74078-3032. FAX: 405-744-7373; Email: CBENDER@VMS.UCC.OKSTATE.EDU.** *OSU and UIC are Equal Opportunity/Affirmative Action Employers.*

RESEARCH ASSOCIATE position available in a program focused on immunology of human melanoma with emphasis on immunotherapy. Background in molecular immunology required to participate in a program to analyze HLA antigen expression by melanoma cells, to develop new types of immunogens for immunotherapy and/or analyze patient's immune response. Send curriculum vitae, names of three references to: **Dr. S. Ferrone, Department of Microbiology and Immunology, New York Medical College, Valhalla, NY 10595.**

RESEARCH & DEVELOPMENT

SENIOR SCIENTIST

Genetic Analysis

Promega Corporation, a world-leading biotechnology company, headquartered in Madison, WI, has an opening for a Senior Research & Development Scientist in Genetic Analysis. Requirements include: Ph.D. in molecular biology or related area and minimum 5 years lab experience. Prefer 2+ years experience in an industrial setting; experience with nucleic acid purification and amplification methods, linkage analysis and/or non-isotopic detection methods; experience handling plant, animal, clinical or forensic materials; and facility with computer-based analyses, especially national genome databases and DNA analysis software.

Promega strives to provide a rewarding work experience for our employees and to provide unparalleled excellence, quality and prosperity in our business. We seek to preserve a balance between work and life activities reflected in our on-site fitness and daycare facilities and comprehensive benefit package. To apply, send a resume with salary requirements to: **PROMEGA CORPORATION, Human Resources (11104J), P.O. Box 7879, Madison, WI 53707-7879.** Equal Opportunity Employer.

Promega

To hear about current employment opportunities, please call our Career Opportunities Line at (608) 277-2539.

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inside IMMULOGIC

Fall 95:

With the financial strength and resources of a well-established organization, ImmuLogic is making its presence felt in the biopharmaceutical community Through a series of strategic moves, we've strengthened and streamlined our organization We've solidified our joint development and commercialization agreement with Hoechst Marion, Roussel, Inc , by signing a letter of agreement for the joint manufacture of ALLERVAX® products. We've also formed a partnership with Schering AG, Germany to develop and market a peptide based multiple sclerosis product. Above all, we've successfully brought the first two products in our ALLERVAX® family of allergy therapeutics to late stage clinical programs. Watch for updates, and more exciting career opportunities as we build on our momentum and move closer to commercialization.

Director - Immunology

In this key position, you will manage and organize our immunology group directing research leading to a definition of T-Cell epitope based products for allergic and autoimmune diseases. Specifically, you will lead a research group to elucidate the mechanism of tolerance induction and the regulation of cytokine synthesis. You will perform administrative and research responsibilities and supervise a minimum of 10 researchers Requires a Ph.D. in Immunology or a related field and a minimum of 12-15 years' experience. Demonstrated excellence in scientific research in immunology is required.

As part of our growing team, you'll enjoy a wide array of professional and personal advantages — from our competitive salary, equity and benefits package, to our entrepreneurial environment. Please send your resume to: **Human Resources, ImmuLogic Pharmaceutical Corporation, 610 Lincoln Street, Waltham, MA 02154.** An equal opportunity employer. Principals.

IMMULOGIC Pharmaceutical Corporation

The clear relationship between business and science

SCIENTISTS/ SR. SCIENTISTS

DRUG DISCOVERY RESEARCH

The R. W. Johnson Pharmaceutical Research Institute conducts research in a number of therapeutic areas including anti-infectives, CNS, dermatology, hematology/oncology, immunology/inflammation, and women's health for the Johnson & Johnson companies: Cilag, Ortho-McNeil Pharmaceutical, Ortho Biotech and Ortho Dermatological. We have immediate openings for two Scientists/Sr. Scientists at our campus-like facilities in CENTRAL NEW JERSEY (midway between New York City and Philadelphia). Excellent communication and interpersonal skills are required to work effectively as members of multi-disciplinary research teams.

Reproductive Medicine

The successful candidate will have a Ph.D. in Biochemistry, Pharmacology, Reproductive Endocrinology or related discipline and at least 2 years of postdoctoral training demonstrating skills in reproductive physiology, receptor biology/signal transduction, cell/tissue culture, molecular biological techniques and small-animal handling. Assay development skills required. Experience in immunocytochemistry and *in situ* hybridization is a plus.

Your primary responsibilities will include the design, execution and analysis of experiments to identify and characterize novel therapeutic targets (and their biological/biochemical responses) in endocrine-related disease states. You will also assist in the development of assays for the evaluation of lead compounds. **(Dept. 504)**

Drug Metabolism

The successful candidate will have a Ph.D. or equivalent, knowledge of immunoassays, and 3+ years of experience performing progressively advanced research. 7+ years' experience, including postdoctoral, required to qualify at the Senior Scientist level.

Responsibilities include assay development, implementation and documentation to support pharmacokinetics studies. In-depth experience is required in RIAs, ELISAs, immunological and other analytical techniques for analysis of proteins/peptides in biological matrices. **(Dept. 505)**

We offer competitive salaries and excellent benefits, including medical/dental insurance, 401(k) plan and our LIVE FOR LIFE Wellness Program with on-site fitness center. Please forward a copy of your resume, including salary requirements, suitable for scanning into our database (i.e., clean/clear, no graphics, and preferably unfolded) to: Dept. # indicated above for the position of your interest, Johnson & Johnson Recruiting, P. O. Box 16597, New Brunswick, NJ 08906-6597.

We are an equal opportunity employer and support diversity in the workplace.

THE R W JOHNSON
PHARMACEUTICAL RESEARCH INSTITUTE
a Johnson & Johnson company

POSITIONS OPEN

POSTDOCTORAL POSITION available immediately to study novel amplified genes in human tumors. Techniques being utilized include microdissection, PCR amplification, fluorescent in situ hybridization (FISH), as well as routine molecular biological techniques. This project includes collaboration with members of the Human Genome Project at The University of Texas Health Science Center at San Antonio. Required: Ph.D. with strong background in molecular biology. Experience with cytogenetics and FISH preferable. Send curriculum vitae, description of research training and experience, and names and addresses of three references to: **S. Gail Eckhardt, M.D., c/o Personnel Department, Cancer Therapy and Research Center, 8122 Datapoint Drive, Suite 600, San Antonio, TX 78229.** *Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION

Available January 1, 1996, at the J. David Gladstone Institute of Cardiovascular Disease in the laboratory of **Dr. Robert V. Farese, Jr.**, to study cellular cholesterol metabolism using a molecular and genetic approach. Methodology involves gene manipulation in mice and cell biology of sterol metabolism. Ph.D. or M.D./Ph.D. with experience in molecular/cellular biology required. Please send curriculum vitae and names of three references to: **HR-C95-062, Gladstone Institutes, P. O. Box 419100, San Francisco, CA 94141. FAX: 415-826-3708.** *Equal Opportunity Employer.*

POSTDOCTORAL RESEARCH ASSOCIATE Environmental Chemist position beginning 1 January 1996, renewable annually pending availability of funds. Experience with GC using FID, PID, ECD, and ELCD detectors. Proficiency with an HP 5890 mass spec and RTE operating system a plus. Salary range $32,000 to $36,000. By 30 November 1995, send curriculum vitae, statement of research interests, and names, addresses, and telephone numbers of five references to: **Dr. Stephen A. Bortone, Institute for Coastal and Estuarine Research, University of West Florida, Pensacola, FL 32514. Telephone: 904-474-3251; FAX: 904-474-3130; Email: sbortone@castaway.cc.uwf.edu.** *University of West Florida is an Equal Opportunity/access/Affirmative Action Employer.*

POSTDOCTORAL MICROBIOLOGIST

USDA, Agricultural Research Service (ARS), National Animal Disease Center (NADC), Ames, Iowa, has an opening for a **MICROBIOLOGIST** to study molecular biology of pseudorabies virus. Candidate will investigate mechanisms of herpesvirus latency. Ph.D degree required. Appointment is for two years and includes benefits. Salary commensurate with experience ($35,578 to $42,641 per annum). Send curriculum vitae and names of three references to: **Andrew Cheung, USDA, ARS, NADC, P. O. Box 70, Ames, IA 50010. Telephone: 515-239-8324; FAX: 515-239-8458.** Applications in response to this advertisement should be marked **5N035.** Applications accepted until position is filled. *USDA/ARS is an Equal Opportunity Employer.*

POSTDOCTORAL POSITION
University of Pennsylvania
School of Medicine

Position available to study cell cycle control of cellular division. Specific areas of study include the control of chromosome segregation using a *Xenopus*-extract based *in vitro* system. Qualified individuals should possess a Ph.D. and have a strong background in biochemistry and experience with molecular biology.

We offer a competitive salary and an excellent benefits package. Send curriculum vitae, research interests and background, and the names and telephone numbers of three references to: **University of Pennsylvania School of Medicine, Attn: Dr. Sandra L. Holloway, 713a Stellar Chance (BRB1), 422 Curie Boulevard, Philadelphia, PA 19104-6069.** *Equal Opportunity Employer.*

POSTDOCTORAL POSITION to study the molecular immunology of human red blood cell antibodies. Cellular and recombinant phage display approaches are utilized to express antibodies and to develop novel inhibitory peptidomimetics. Applicants should send a curriculum vitae and three references to: **Dr. Don L. Siegel, Room 207, J. Morgan Building, University of Pennsylvania, Philadelphia, PA 19104-6082. Email: siegeld@mail.med.upenn.edu.** *University of Pennsylvania is an Equal Opportunity/Affirmative Action Employer.*

POSITIONS OPEN

Agricultural Research Service — United States Department of Agriculture

RESEARCH ASSOCIATE–The USDA, Agricultural Research Service (ARS), is seeking a candidate to work on wheat molecular biology and transformation. Candidate to join a project working to bioengineer wheat characteristics and to improve wheat transformation technology. Experience in molecular biology and plant tissue culture desired. Position is a temporary two-year appointment with full benefits. Salary range is $37,087 to $44,450, commensurate with qualifications. Send letter and curriculum vitae to: **Dr. Olin D. Anderson, USDA, ARS, WRRC, 800 Buchanan Street, Albany, CA 94710. Telephone: 510-559-5773; Email: oandersn@pw. usda.gov.** Position is open until filled. *The USDA is an Equal Opportunity Employer.*

ASSOCIATE SCIENTIST
AND POSTDOCTORAL POSITIONS

To work in the laboratory of **Dr. Kenneth M. Baker,** on G-protein receptor mediated cellular growth and the role of tyrosine phosphorylation in this process. Emphasis will be placed on the angiotensin II (type AT_{1A}) receptor in cardiovascular cells and stably transfected cell lines, to define the mechanisms of coupling to JAK-STAT and other signaling pathways and the importance of these events in gene regulation and cellular growth. Other areas of investigation include the molecular regulation of angiotensin receptors and of renin-angiotensin system genes in cardiac cells. A Ph.D. and/or M.D. is required with a background and experience in biochemistry, molecular and cellular biology. Salary will be commensurate with education and experience. Send letter of interest along with curriculum vitae and three letters of reference to: **Geisinger Human Resources (KMB), 100 North Academy Avenue, Danville, PA 17822-1529.** *Equal Opportunity Employer. Minority/Female/Disabled/Veteran.*

POSTDOCTORAL POSITION
IMMUNOLOGY

Position available immediately to study the maternal reproductive tract T lymphocyte biology in normal and abnormal pregnancy in a murine model. Position suited to a recently graduated postdoctoral fellow with a strong interest in cellular and molecular immunology. Animal work involved. Unique setting in a private research setting with NIH funding. Please send a curriculum vitae, a brief letter of research interests, and three reference letters to: **Kent D. Heyborne, M.D., Swedish Medical Center, 501 East Hampden Avenue, Englewood, CO 80110.**

SENIOR RESEARCH ASSOCIATE

The City of Hope invites applications for a position to manage a transgenic mouse facility for research programs at the NCI-Designated City of Hope Cancer Center and Beckman Research Institute. The facility is associated with a research laboratory with substantial experience in the techniques. Work involves (1) microinjection of fertilized ova with DNA, injection of blastocysts with embryonic stem cells, surgical techniques of ova transfer, and (2) culture of embryonic stem cells, involving routine maintenance, transfection with DNA, and picking and freezing of recombinant clones. Significant previous experience in (1) is essential, a Ph.D. is not essential. This position will be working in **Dr. Jeff Mann's** lab, Division of Biology, Beckman Research Institute of the City of Hope. Submit application, with curriculum vitae and three addresses for letters of reference, to: **City of Hope, Human Resources, Attn: JM, 1500 East Duarte Road, Duarte, CA 91010-3000. FAX: 818-301-8448; Email: jyasmineh@smtplink. coh.org.** *Equal Opportunity Employer.*

SENIOR RESEARCH ASSOCIATE

The Department of Neurology, Columbia University College of Physicians and Surgeons, seeks a **SENIOR RESEARCH ASSOCIATE** to assist in laboratory work in neurogenetics. Responsibilities will include DNA sequencing and sequence analysis, mutation analysis, RNA and protein extraction and assay. M.D. or Ph.D. required, with at least three years of postdoctoral experience in the necessary techniques. Send curriculum vitae to: **Torbjoern G. Nygaard, M.D., Department of Neurology, Columbia University, 710 West 168th Street, New York, NY 10032.**

Columbia University is an Affirmative Action/Equal Opportunity Employer.

POSITIONS OPEN

POSTDOCTORAL POSITIONS
Roswell Park Cancer Institute
Department of Human Genetics
with Thomas B. Shows, Ph.D. and
Pieter J. de Jong, Ph.D.

Positions available for Ph.D./M.D. in human genome research including projects involving: efficient construction of sequence ready clone sets; analysis of conserved repetitive DNA; application of recombination-based cleavage methods for chromosome analysis and genome manipulation; transcript mapping; positional cloning of disease loci; sequencing the genome; and improvements in large-insert cloning technology.

Experience in molecular genetic and genome technology is required. Please send curriculum vitae, a description of your research experience and future interests, and names and telephone numbers of three references to: **Chair, Department of Human Genetics, Roswell Park Cancer Institute, Buffalo, NY 14263.** *Roswell Park is a Minority/Female/Disabled/Veteran Affirmative Action Employer.*

GCG

TECHNICAL SUPPORT REPRESENTATIVE

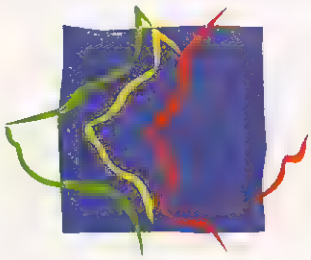
The Genetics Computer Group, Inc. (GCG) is seeking an additional member for its technical support staff to provide assistance to the world-wide community of biologists that use the GCG Wisconsin Sequence Analysis Package. The ideal candidate will have an advanced degree in molecular biology/biochemistry or related field, computer experience, and strong interpersonal and communication skills. Familiarity with the Wisconsin Sequence Analysis Package will be an advantage. Salary is negotiable. GCG offers a full range of benefits and help with relocation costs if applicable. For more information visit GCG's homepage at: **http://www.gcg.com.** Send curriculum vitae with references to: **Genetics Computer Group, Inc., 575 Science Drive, Madison, WI 53711. Telephone: 608-231-5200.** *Genetics Computer Group, Inc. is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITION available to participate in studies on the structure, function and regulation of components of signal transduction pathways. Prefer applicants with experience in molecular cloning and recombinant DNA techniques. Send curriculum vitae to: **Dr. Zahi Damuni, Associate Professor, Department of Cellular and Molecular Physiology, The Pennsylvania State University College of Medicine, 500 University Drive, Hershey, PA 17033. Telephone: 717-531-4195; FAX: 717-531-7667.** *For your health, Hershey Medical Center is a smoke-free campus. We are an Equal Opportunity/Affirmative Action Employer and encourage minority and female applicants to apply.*

POSTDOCTORAL POSITION available. The NIH National Center for Human Genome Research has a position available in the Laboratory of Genetic Disease Research under the supervision of **Dr. Leslie Biesecker.** The successful applicant will study the molecular genetics of Proteus syndrome and related disorders. Please contact **Dr. Biesecker by Telephone: 301-402-2041; FAX: 301-402-2170; Email: leslieb@helix.nih.gov;** or post: **49 Convent Drive, MSC 4470, Bethesda, MD 20892.** *The NIH is an Equal Opportunity Employer. Women and minorities are encouraged to apply.*

POSTDOCTORAL POSITION available for mechanistic studies of DNA helicases using transient kinetics (stopped-flow, quenched-flow), biochemical and biophysical approaches. Send curriculum vitae and have three letters of reference sent to: **Prof. Timothy M. Lohman, Biochemistry and Molecular Biophysics, Washington University School of Medicine, Box 8231, 660 South Euclid Avenue, St. Louis, MO 63110.**

POSTDOCTORAL POSITION available to carry out the sequencing, analysis, and expression of genes associated with polyketide antibiotic biosynthesis in *Streptomyces*. Previous experience with molecular biology required; previous experience with *Streptomyces* highly desirable. Send curriculum vitae and the names and telephone numbers of three references to: **Professor Ronald Parry, Department of Chemistry, Rice University, P. O. Box 1892, Houston, TX 77251.**



RESEARCH GRANTS

Offered by

THE FONDATION
PIERRE RICHARD DICK -VIRBAC

under the aegis of the Fondation de France

IF YOUR RESEARCH CONCERNS INFECTIOUS DISEASES OF VIRAL, BACTERIAL OR PARASITIC ORIGINS

"Support actions in the area of research and information in favour of Animal Health, with a view to improving Man's Health and Environment."

All qualified groups or individuals are invited to submit applications for research grants to undertake basic or applied medical and veterinary research in the field of:

- **New vaccines or new methods of vaccination** within the framework **of infectious and transmissible diseases aiming to benefit both human beings and animals.**

Particular attention will be given to projects displaying positive impact on the environment and developing countries, clear and economically acceptable objectives, and collaboration between **Veterinary and Human Research.**
A minimum of **two Grants of $ U.S. 25,000 each** will be awarded for one year, beginning in **June 1996.**

The deadline for submission of your proposal is **January 31, 1996.**

Orientation Committee
Fondation Pierre Richard Dick - Virbac
B.P. 305, 06514 CARROS Cedex, FRANCE
Tel: (33) 92 08 72 49 Fax: (33) 92 08 72 82

PLEASE CONTACT US QUICKLY FOR AN APPLICATION FORM

POSTDOCTORAL POSITIONS

APOPTOSIS AND CELL ADHESION RESEARCH

Astra is a pharmaceutical company in a phase of rapid growth. The company´s operations, which are highly international, consist in research, production and marketing of pharmaceuticals through subsidiaries, agents and licensees all over the world. The number of employees is about 14,400, of whom 5,300 in Sweden. Sales in 1994 amounted to SEK 28 bn (USD 3.65 bn).

ASTRA ARCUS AB
Astra Arcus, a research company in the Astra Group, is engaged in R & D in primarily two areas – CNS and Infection. Astra Arcus employs some 430 people and the company is located in Södertälje, Sweden, 35 kilometres south of Stockholm.

Two postdoctoral research positions are immediately available for studies on apoptosis and cell adhesion in relation to neuroimmunological diseases. Both positions are at the department of Immunology, Preclinical R&D, Astra Arcus AB, Sweden.

Suggested topics of apoptosis research are:
-regulation of apoptosis in autoreactive T lymphocytes
-importance of organ-specific survival factors
-pathology of apoptotic mechanisms

A suggested topic of cell adhesion research is to investigate the importance of integrins in relation to neurodegenerative disorders.

The postdoctoral positions will work in a team of researchers engaged inexplorative research in the field of neurodegenerative disorders. Your work will be related to newly initiated projects and provide opportunities for a creative individual. Collaboration with external academic groups as well as publication of results is encouraged. The offered positions are for 2 years.

Further information can be obtained from Dr. Carl Harald Janson and Dr. Kjell Stenberg, +46 8 553 273 47 and +46 8 553 260 93; fax +46 8 553 270 60.

We would like your application with curriculum vitae before November 10, 1995 to Astra Arcus AB, Human Resources, Louise Bergman, S-151 85 Södertälje, Sweden.

ASTRA
ASTRA ARCUS

POSITIONS OPEN

McLaughlin Research Institute for Biomedical Sciences

POSTDOCTORAL ASSOCIATE
McLAUGHLIN RESEARCH INSTITUTE

POSTDOCTORAL POSITIONS are available to study the molecular genetics of molecular motors, using the mouse as a model system. MRI is an expanding basic research institute that is devoted to the mouse as an experimental model. The Institute consists of highly interactive research groups housed in a new building with state-of-the-art transgenic and gene targeting facilities. Send curriculum vitae with names and addresses of three references to:

John A. Mercer, Ph.D.
McLaughlin Research Institute
1520 23rd Street South
Great Falls, MT 59405
Email: umbjm@gemini.oscs.montana.edu

An Equal Opportunity/Affirmative Action Employer.

POSTDOCTORAL POSITION
VASCULAR BIOLOGY-
SMOOTH MUSCLE CELL CYCLE

Available immediately to investigate transgenic models of smooth muscle proliferation and altered differentiation, with additional laboratory emphasis on local gene delivery. Research involves the mechanisms of smooth muscle cell cycle control *in vitro* and *in vivo*. Other studies in the laboratory involve local placement of vascular cell-modulating genes to create "local transgenic" models. Prior experience in molecular biology is desired. Competitive stipend and an opportunity to make important contributions are available. Contact: **Keith L. March, M.D., Ph.D., Krannert Institute of Cardiology, 1111 West 10th Street, Indianapolis, IN 46202-4800. Telephone: 317-630-8257; FAX: 317-274-9697; Email: march@kimail.dmed.iupui.edu.**

POSTDOCTORAL POSITIONS
ARKANSAS CANCER RESEARCH CENTER

POSTDOCTORAL POSITIONS are available as part of a multidisciplinary program in B cell neoplasia at the University of Arkansas for Medical Sciences. Specific areas include identification of genes associated with cell-mediated cytolysis for gene and immunotherapy, and regulation of tumor growth and apoptosis by cytokines and oncogenes. Ph.D.s and/or M.D.s with experience in molecular biology, cell biology and/or immunology are encouraged to apply. Send curriculum vitae and names of three references to: **Dr. Jacki Kornbluth, Division of Hematology/Oncology, University of Arkansas for Medical Sciences, 4301 West Markham–Slot 508, Little Rock, AR 72205. FAX: 501-686-8165; Email: jkornbluth@acrc.uams.edu.** *Affirmative Action/Equal Opportunity Employer.*

PROTEIN BIOCHEMISTRY

A **POSTDOCTORAL POSITION** is available for the preparation, modification, and crystallization of a variety of motor and structural proteins. Enzymology background helpful. The successful candidate would work closely with a group of protein crystallographers. Training in protein crystallization (as well as other aspects of structure determination, if desired) would be provided. Send curriculum vitae and three reference letters to: **Dr. C. Cohen, Rosenstiel Basic Medical Sciences Research Center, Brandeis University, Waltham, MA 02254-9110. FAX: 617-736-2419; Email: ccohen@binah.cc.brandeis.edu.**

POSTDOCTORAL POSITIONS IN
MOLECULAR AND CELLULAR BIOLOGY

To study the bombesin-like peptides (BLP). Projects include: 1) Cloning and characterization of bombesin-like peptide receptors; 2) Characterization of new mammalian bombesin-like peptides; 3) Characterization of the role of BLP in fetal monkey lung development. Methods include a mix of molecular biology, protein purification and *in vivo* physiologic models. Send curriculum vitae and names of three references to: **Eliot Spindel, M.D., Ph.D., Division of Neuroscience, Oregon Regional Primate Research Center, 505 NW 185th Avenue, Beaverton, OR 97006.** *Equal Opportunity Employer.*

GRADUATE STUDENT FELLOWSHIPS
IGPP/UCLA

The Institute of Geophysics and Planetary Physics (IGPP) at UCLA invites applications for **GRADUATE FELLOWSHIPS** for 1996–1997, two to be awarded in each of three centers of excellence: Center for the Physics and Chemistry of the Planets; Center for Earth System Research; and the Space Science Center.

In addition to support provided fellows by UCLA Departments, IGPP will grant each fellow a stipend of $5,000 for the first year of graduate study, with no teaching or other duties. Stipends may be used for any purpose the fellow deems appropriate: travel, books, computers, etc. Fellows are included in a select group of graduate students invited to interact with IGPP faculty in weekly seminars and luncheons.

Applicants should plan to carry out doctoral research under the direction of an IGPP faculty member. Faculty are members of the UCLA Departments of Earth and Space Sciences, Chemistry and Biochemistry, Atmospheric Sciences, Physics and Astronomy, Mathematics, and the Molecular Biology Institute.

Fellowship applicants must apply by 15 January 1996 to IGPP. Applicants must also apply to an appropriate academic department for admission to UCLA.

For application forms and a list of IGPP members, contact: **IGPP Fellows Committee, 3845 Slichter Hall, UCLA, Los Angeles, CA 90095-1567.**

For further information please telephone **310-825-1580.**

UCLA is an Equal Opportunity Affirmative Action Employer.

POSTDOCTORAL POSITIONS
Structural Basis of Neurobiology

Postdoctoral positions are available on a training grant in Neurobiology based in the Department of Anatomy and Neurobiology at Boston University School of Medicine. The training, in an active research environment, is designed to strengthen the trainees' knowledge of the structural and functional organization of the nervous system. Training is carried out under the direction of one of the eighteen participating faculty members, who have a wide range of interests.

For further information please contact:

Dr. Alan Peters
Department of Anatomy and Neurobiology
Boston University School of Medicine
80 East Concord Street
Boston, MA 02118
Telephone: 617-638-4200
FAX: 617-638-4216

Boston University is an Equal Opportunity/Affirmative Action Employer.

A **POSTDOCTORAL POSITION** is available in bacteriophage genetics to study the takeover of E. coli by T4 phage, using a multidisciplinary approach. This virus has now been completely sequenced; the goal of this project is to identify and characterize the products of genes predicted to alter host metabolism, and their host targets. Applicants should possess a related Ph.D. degree and broad and extensive experience with bacterial viruses and molecular biology techniques, including DNA sequencing, cloning and expression of host-lethal genes, and purification and biochemical characterization of proteins. We are looking for someone with the desire and ability to work closely with undergraduate students. Review of applications begins November 1, 1995, and will continue until finalists are chosen. For detailed job announcement **Telephone: 360-866-6000 x6361.** Submit curriculum vitae and names of three references to: **Dr. James Neitzel, Lab I, The Evergreen State College, Olympia, WA 98505. FAX: 360-866-6794; Email: neitzelj@elwha.evergreen.edu.** *The Evergreen State College is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL POSITIONS available to study genes that determine the yeast replicative life span, their human homologs and age-responsive promoter elements (*Genetics*, 91:35, 1993; *J. Biol. Chem.*, 269:15451, 1994; *J. Biol. Chem.*, 269:18638, 1994). Experience in molecular techniques essential and in yeast genetics helpful. Send curriculum vitae with bibliography and names, telephone and fax numbers of three references to: **Dr. S. Michal Jazwinski, Department of Biochemistry and Molecular Biology, Louisiana State University Medical Center, 1901 Perdido Street, New Orleans, LA 70112.** *Affirmative Action/Equal Opportunity Employer.*

POSTDOCTORAL POSITION
Molecular Cytogenetic Studies of Prostate Cancer

An immediate position is available to characterize specific molecular genetic and molecular cytogenetic alterations important for the initiation and progression of prostate carcinoma. The position is funded for one year with potential for extension into a second year. Potential studies include chromosomal deletion mapping by *in situ* hybridization and loss of heterozygosity analysis, DNA mutation screening and sequencing, and somatic cell genetic experiments. A strong background in molecular genetics and/or molecular cytogenetics is desirable. Applicants should mail or FAX their curriculum vitae and the names and FAX numbers of three qualified references to: **Dr. Robert B. Jenkins, Division of Laboratory Genetics, Mayo Clinic, 200 First Street SW, Rochester, MN 55905. FAX: 507-284-0043.** Applications will be accepted until October 30, 1995.

A **POSTDOCTORAL POSITION** is available to conduct a protein structure and function analysis of a site-specific DNA endonuclease. Research project will include isolation of mutant alleles using genetic techniques and characterization of mutual proteins. Strong background in biochemistry and molecular biology required. Send curriculum vitae and three letters of recommendation to: **Dr. F. Gimble, Institute of Biosciences and Technology, Texas A&M University, 2121 West Holcombe Boulevard, Houston, TX 77030.** *Affirmative Action/Equal Employment Opportunity Employer.*

PRE- AND POSTDOCTORAL FELLOWSHIPS
MOLECULAR PARASITOLOGY

The Molecular Parasitology Training Program in the Division of Biological Sciences, University of Georgia has immediate openings for **PRE- AND POSTDOCTORAL FELLOWS** with an interest in the Molecular and Cell Biology of Parasites and Vectors. This NIH-sponsored program offers training in parasitology with emphasis in the areas of molecular biology, biochemistry, immunology, cell biology, and vector biology. With a staff of 20 faculty participants from the University of Georgia and the Centers for Disease Control, this program combines the expertise of classical and modern parasitologists and immunoparasitologists with that of molecular biologists, cell biologists, and biochemists to provide comprehensive and modern training in molecular parasitology. *Funding is restricted to U.S. citizens or permanent residents.* For further information contact: **Molecular Parasitology Training Program, Department of Cellular Biology, University of Georgia, Athens, GA 30602.** *The University of Georgia is an Equal Opportunity/Affirmative Action Employer.*

POSTDOCTORAL FELLOWSHIP/RESEARCH INSTRUCTOR positions available in two laboratories: **Mark Boothby, M.D., Ph.D.:** Transcription and signal transduction factors involved in lymphocyte differentiation and responses to IL-4; IL-4 related transgenes. **Geraldine Miller, M.D.:** FGF function and signaling in transplantation, vascular disease, and autoimmunity. Experience in molecular biology required. Background in immunology or signal transduction desirable. Please address statement of interests, curriculum vitae, and references to the investigator: **Dr. Mark Boothby, Department of Microbiology and Immunology, Vanderbilt University, Nashville, TN 37232-2363. Dr. Geraldine Miller, Department of Medicine, Vanderbilt University, Nashville, TN 37232-2605. Email: millergg@ctrvax.vanderbilt.edu.**

Biotech company in Ventura, California seeks experienced professionals for the following positions: **Clinical Database Specialist.** Provide clinical data review/development/implementation of EDDS system into data review workflow—requires knowledge of clinical trials process/clinical terminology. SQL DB experience, biological science or computer science degree, two plus years of experience in clinical data management or biotech company. **Safety Assistant.** Take adverse event information via telephone, enter data into DB, assist with MGDF and BDNF projects, must be RN with nephrology or acute disorders experience. **Clinical Records Coordinator.** Initiate study specific CRIC and CARS DB. Process clinical documents into "CARS" system, provide CRIC document inventory reports, handle "Special Processing Issues"/Clinical Trials Conduct, familiar with relational DB applications. **United Staffing Service, 325 Rolling Oaks Drive, Suite 105, Thousand Oaks, CA 91361. Contact Monicaur Barbara. Telephone: 805-371-8100; FAX: 805-494-6763.**

Department of Biological Sciences

CHAIR

IN

PROTEIN STRUCTURE STUDIES

Applications are invited for this newly established Chair, available now, to lead a new Research Group working on the determination of high resolution protein structure using X-ray diffraction, within the context of addressing important biological questions. The Department has built a strong research base in virology, microbiology, plant biochemistry and in animal developmental biology – with a focus on molecular biology, cell biology and biochemistry, and has recently set up Research Groups in Ecosystems Analysis and in Molecular Medicine.

Salary will be within the Professorial Range, minimum £31,999 pa.

Informal enquiries are welcome and may be made to Professor M.A. McCrae (Tel: 01203 523524; Fax: 01203 523568; e-mail: malcolm@dna.bio.warwick.ac.uk).

Further particulars can be obtained from the Personnel Office, University of Warwick, Coventry CV4 7AL (01203 523627) quoting Ref. No. 10/A/95 (please mark clearly on envelope).

Closing date: 30th November 1995.

UNIVERSITY OF WARWICK

The University of Warwick is an exempt charity dedicated to excellence in teaching and research and is an Equal Opportunities Employer

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Request for Proposals From
The International Spinal Research Trust

The ISRT is a grant giving charity (number 281325) with the purpose of funding research aimed at resolving the non or partial functioning of the injured spinal cord.

The field of spinal cord repair has developed to the stage at which we now have demonstrable regeneration of damaged nerve fibres in the adult mammalian cord. This represents a truly great advance that is the foundation of all our research and, even more importantly, the associated technologies that will help us progress are now maturing. Accordingly we feel that we have sufficient knowledge to plan a research strategy which will guarantee genuine progress. At the moment, we can only look to phase one of a strategic programme, but we are confident that this will take us forward to a second phase where we can begin to address how to take the work on to the clinic.

To reach this point it is recommended that ISRT funding is, from now on, restricted to projects that are conducted in adult mammalian spinal cord and concentrated on 3 main research foci:

- **early trauma and inflammation:** prevention of the generation of obstacles to future regenerative repair, including aspects of scarring
- **trophic stimulation of neurite outgrowth:** identification of the pattern and possible temporal cascade of the different factors required to achieve long-distance regeneration
- **CNS inhibitory molecules:** masking of intrinsic mechanisms that are inhibitory to axonal regrowth in the CNS

Besides these three main foci, ISRT will support a complementary programme to address the hurdles that prevent a proper evaluation of the progress made on each project.

- *A programme to develop underpinning technologies.* The development of assessment models: both behavioural models and models that closely mimic the lesion in man.
- *Education and communication.* Educating young scientists in relevant technologies and encouraging exchange visits between laboratories.

The Trust does not, at this time, have the resources to begin every aspect of this three year programme. Accordingly applications are invited for projects focusing on the **early trauma and inflammation.** Applicants should submit a letter of intent (c. two sides) summarising the proposed research and background of the laboratory. An approximate budget and the curriculum vitae of the Principal Investigator(s) should be appended. Awards will average up to £250,000 over three years and, following a preliminary review, detailed proposals will be requested from a limited number of applicants. Deadline for letters of intent is 30th November 1995. Successful applicants will be approached by ISRT for detailed plans.

Address letters of intent to: The International Spinal Research Trust, Unit 7 Bramley Business Centre, Station Road, Bramley, Guildford, Surrey GU5 0AZ

UNIFOB

BERGEN UNIVERSITY RESEARCH FOUNDATION

DIRECTOR
MOLECULAR MARINE BIOLOGY LABORATORY

The Bergen University Research Foundation is seeking an outstanding scientist as director of a new Molecular Marine Biology Laboratory at the Bergen High Technology Centre.

The Molecular Marine Biology Laboratory is part of an initiative taken by the Research Council of Norway to strengthen basic marine research and will conduct research oriented towards marine organisms and their interaction with their environment. The research programmes should therefore include Molecular Marine Ecology and Molecular and Cell Biology of Marine Organisms with the following topics as possible research areas; Interactions between microorganisms, and between microorganisms and higher taxa; Development of the immune system; Responses of organisms to environmental signals; Differentiation and developmental biology; Reproductive biology; Production of beneficial chemicals and toxins from the marine environment; Description of ecosystems in the polar environment.

It will be a top priority to recruit an international scientific staff and initiate new research networks within molecule marine biology and marine biotechnology. The Molecular Marine Biology Laboratory will be part of a Large Scale Facility for marine research funded by the European Union 4. framework programme for training and mobility of researchers. Grants will be available to cover travel, subsistence and user fees for visiting scientists.

The research activities will be organized in separate research groups or programmes. Laboratory core facilities including access to special laboratories for research on aquatic organisms will be available January 1996. Selection of group leaders, research programmes and installation of necessary scientific equipment is planned to start during the first half of 1996. Funding will be available to start three to four research groups in 1996. The laboratory may house a maximum staff of 80 with a core staff of 40 including group leaders.

The director will be in charge of the work to establish the Molecular Marine Biology Laboratory. The director will also, in collaboration with a Scientific Advisory Committee, coordinate the scientific programme and select group leaders. The appointee may include own research activities in the research programme as a director's group. The laboratory will be organized as a division of the Bergen University Research Foundation with a separate administrative staff and board of directors. The director will initially be engaged for a period of six years. The engagement may be prolonged for a maximum of three years. Salary will be negotiable.

Deadline for applications is 20 November 1995.

For further information please contact

Research advisor Ivar Lossius, e-mail: ivar.lossius@fa.uib.no or Fax: +47 5558 4981, Tel: +47 5598 4995

To apply please send your CV quoting ref.no. 93/2629, to:

Liv-Grethe Gudmundsen
Bergen University Research Foundation
SEP-Section for Project Services
Professor Keysers gt.8

KUWAIT UNIVERSITY
Faculty of Science

Faculty of Science at Kuwait University seeks qualified candidates in Botany and Microbiology (Plant fine structure and Anatomy, Phycology (Marine Botany), Plant Ecology (vegetation Ecology), Plant Physiology (growth hormones and regulators) with a strong commitment to high quality teaching and research for appointment at the positions of Associate Professor and Assistant Professor.

Required Qualifications:

- Ph.D. or its equivalent, in the required area
- Research experience and publications
- University teaching experience
- Excellent knowledge of English

Kuwait University offers:

- TAX FREE Salary (approximate ranges: Associate Professor - KD 875-1035 and Assistant Professor - KD 680-840) monthly depending on years of experience and number of accompanying dependents (spouse and children under 18). [1 KD equivalent to approximately US $3.5]
- Fringe benefits which include: furnished air-conditioned accommodation, annual air tickets, free National Health Medical Care, paid mid-term holidays and summer vacations and gratuity.
- New appointees can apply for financial support for research projects.
- Excellent academic environment.
- Contracts effective from September, 1996, for a two year initial period

Application should be accompanied by:

- A complete C.V, including: Mailing address including phone, fax, and e-mail. Academic qualifications, teaching, research and professional/work experience,and list of publications in professional journals.
- Names and addresses of three persons well-acquainted with the academic and professional work of the applicant.
- An English translation of all documents in other languages should be enclosed.
- For appointment to the position of Assistant Professor, a very good performance in the B.Sc. is required (minimum GPA 3 points out of 4). Please send copies of all transcripts.

Applications should be sent by express mail/courier service to:

The Dean, Faculty of Science
Kuwait University, P.O. Box 5969
Safat, 13060, KUWAIT

For inquiries use:

Fax: (965) 4847054 or (965) 4836127
E-Mail: AZZA@KUCOI.KUNIV.EDU.KW.

Closing date for receipt of applications: November 30, 1995.

European Science Education

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SCIENCE
COVERS THE WORLD

University of Kuwait
Faculty of Medicine
HEALTH SCIENCE CENTER

Pre-clinical and Clinical Appointments:
Applications are being a accepted for the following appointments in the Faculty of Medicine:

ANATOMY:
Professor/Associate Professor/Assistant Professor
- Human Gross Anatomy
- Neuro-anatomy
- Microscopic or Developmental Anatomy

BIOCHEMISTRY:
Professor/Associate Professor/Assistant Professor
- Nucleic Acid Specialist(s)
- Protein Biochemist(s)
- Membrane Biochemist(s)
- Endocrinology oriented Biochemist

COMMUNITY MEDICINE & BEHAVIORAL SCIENCES:
Professor/Associate Professor/Assistant Professor
- Social Psychologist
- Environmental & Occupational Health (Applicants should hold an M.D. as well as a Ph.D. or Dr.P.H. degree in Occupational/ Environmental Health or Occupational/ Environmental Epidemiology. Minimum of two year's teaching as well as research experience is required.)

MEDICINE:
Professor/Associate Professor/Assistant Professor
- Therapeutic
- Clinical Haematology
- Infectious Diseases
- Respiratory Medicine

NUCLEAR MEDICINE:
Professor/Associate Professor/Assistant Professor
- Clinical Nuclear Medicine Physicians
- Nuclear Medicine Physicists
- Radio Immunology Scientist

OBSTETRICS & GYNECOLOGY

Professor and Chairman	- Reproductive Medicine
Professor	- Gynecological Oncology
Associate Professor	- Perinatal Medicine & Gynecological Urology
Assistant Professor	- Obstetrics & Gynecology

PEDIATRICS:
Professor/Associate Professor/Assistant Professor
- Pediatric Neurology
- Pediatric Nephrology
- Pediatric Allergy & Clinical Immunology
- Pulmonary Medicine
- Pediatric Critical Care Medicine
- Neonatologist
- Emergency Medicine
- Pediatric Cardiology
- Clinical Genetics

PRIMARY CARE:
Professor/Associate Professor/Assistant Professor
- Family Practice
- General Practitioner

(MRGCP or equivalent, knowledge of Arabic language will be an advantage)

RADIOLOGY:
Professor/Associate Professor/Assistant Professor
- Consultant Radiologists

(interest in X-ray mammography and breast cancer, and/or MRI imaging and/or Interventional Radiology will be an added recommendation)

SURGERY:

Professor	-Vascular Surgery
Professor	- Transplant
Professor/Associate/Assistant	- General Surgery
Professor/Associate	- Orthopaedics
Professor/Assistant	- Urology
Professor/Assistant	- Anaesthesia

REQUIREMENTS FOR APPOINTMENT:

Qualifications: Applicants should possess a Ph.D. or an equivalent high professional qualification, i.e. FRCS/MRCP/MRCOG/ MRCPATH/MD/American Board in their respective specialty and have conducted and published research in their field. Professors should have at least 14 years experience, 4 as an Associate Professor or its equivalent. Associate Professors should have at least 9 years experience, 4 as an Assistant Professor or its equivalent.

Benefits:

Conference attendance. Gratuity. Furnished accommodation (electricity and water free of charge). Free medical treatment in Kuwait. Free annual round trip air tickets from country of citizenship or permanent residence for self and family, up to three dependent children. Baggage and freight allowance. Education fees for maximum three children in Kuwait from elementary through high school. No taxation. Currency is transferable without restriction. 60 days paid annual leave.

Method of Application:
Curriculum vitae in duplicate which should include the names of three referees; personal particulars; qualifications with dates, career history with dates, teaching experience, research accomplishments and, where appropriate, clinical experience, should arrive no later than November 30th, 1995 to:

The Vice-Dean Administration (Recruitment Office)
Faculty of Medicine
Kuwait University
P.O. Box 24923
13110 Safat, KUWAIT
or Fax: 965 531 8454

Cell Biologist
Department of Biological Sciences
Dartmouth College

The Department of Biological Sciences at Dartmouth College seeks applicants for a tenure track position at the level of Assistant Professor. We expect the successful candidate to establish and supervise an independent research program that will attract extramural funding and provide research training for graduate and undergraduate students. The successful applicant will join a group of faculty whose research involves cellular, genetic, and biochemical approaches to studying problems in a number of model systems. Candidates should have a Ph.D., (or equivalent,) and at least two years of relevant postdoctoral experience. Candidates should expect to participate in teaching cell biology or biochemistry to undergraduates as well as a graduate course in their area of research specialization.

Please send a current curriculum vitae, a statement of research and teaching interests, and arrange to have three letters of reference sent under separate cover to:

Cell Biologist Search Committee
Department of Biological Sciences
Dartmouth College
6044 Gilman Hall
Hanover, NH 03755-3576

Although materials can be submitted by FAX (603-646-1347), please note that the original documents are ultimately required. Application review will begin on December 1, 1995. Women and members of minority groups are strongly encouraged to apply.

Dartmouth College is an Equal Opportunity/ Affirmative Action Employer.

Director, Clinical Pathology Unit
Institute for Human Gene Therapy

University of Pennsylvania

The Institute for Human Gene Therapy, at the University of Pennsylvania as an opportunity for an individual to direct a service and research laboratory involved in histopathology, morphology, and assays of immune function. This person will have proven scientific and management skills, strong supervisory skills, and the ability to assist and advise investigators and researchers in the development of projects involving analysis of tissues by immunocytochemistry. Responsibilities include supporting toxicology studies under GLP as well as basic research, developing new technologies in cellular analysis of gene expression, and managing full laboratory staff. Qualifications include a Ph.D. in biologic sciences, three to five years of research experience with two to four years supervisory experience,.We offer a competitive salary and a comprehensive flexible benefits package including health and dental coverage. For consideration send resume to **James M. Wilson, M.D., Ph.D., Institute for Human Gene Therapy, University of Pennsylvania Health System, 640 Maloney, 3400 Spruce Street, Philadelphia, PA 19104-4283.**

The University of New Mexico
Health Sciences Center

MOLECULAR IMMUNOLOGIST/BIOLOGIST

Research Associate

The University of New Mexico Health Sciences Center, Epidemiology and Cancer Control Program and the Department of Cell Biology has an immediate opening for a Research Associate II to investigate the molecular basis of human papillomavirus (HPV) persistence. Of current interest are the elucidation of specific HLA risk/protective mechanisms of HPV 16-associated disease progression, investigations of direct immunological knockout/mimicry, and general immune response phenomenon. Required: Recent Ph.D. in the biological sciences and one year of directly related experience. Experience in molecular and immunologic techniques including work with cell culture, nucleic acids, and proteins. Training in recombinant DNA technology and protein purification. Position is dependent upon grant funding which is currently available for two years with possible continuation. This position is in the laboratory of Dr. Cosette Wheeler at the UNM Cancer Center. To apply send a curriculum vitae and cover letter of interest with reference to requisition number 956050B, to the **Human Resources Office at the Health Sciences Center, Med. Bldg. 2, University of New Mexico Health Sciences Center, Albuquerque, NM, 87131,** before 5 pm on 10 January 1996. *The University of New Mexico is an Affirmative Action/Equal Opportunity Employer and Educator.*

GLOBAL CAREER OPPORTUNITIES

UNIVERSITY OF GLASGOW

Department of Medical Genetics
POST-DOCTORAL
RESEARCH POSITIONS

Two post-doctoral positions are available to study the involvement of TGFß and its receptors in early mammalian development, specifically in primitive haematopoiesis and vasculogenesis. The approaches taken will be extensive molecular developmental analysis of existent. TGFß1 knock out mice (*Development* 121, 1845-1854, 1995), and the generation and analysis of TGFß receptor transgenic mice using a binary tetracycline-inducible system. Applicants should be experienced in post-implantation murine embryology, transgenic mouse production and analysis, or be expert molecular biologists familiar with gene transfer and expression technology. They would join an established group of 10 people working on related projects.

The posts are both funded by the MRC, one post available for 16 months and one post available for 3 years) and salaries would be on the RA1A scale, dependent on age and experience.

Applications, in the form of a full CV, with the addresses and telephone numbers of three referees, should be sent to Dr. Rosemary Akhurst, Department of Medical Genetics, Glasgow University, Royal Hospital for Sick Children, Yorkhill, Glasgow G3 8SJ. Tel: 0141-201-0365; Fax 0141-357-4277; email gpva06@udcf.gla.ac.uk. Closing date for applications: 1st December 1995. Ref: 8936/S. An equal opportunities employer.

The University of Glasgow is an exempt charity dedicated to excellence in teaching and research.

Middle East
Desalination Research Centre

An institution conceived in the context of the Middle East Multilateral Peace Process

The Governments of the United States of America and the Sultanate of Oman on behalf of the Water Resources Working Group of the Multilateral Phase of the Middle East Peace Process seek qualified candidates for the position of

Centre Director

to lead a process of institutional development

Located in Muscat, Sultanate of Oman, the Centre is a new establishment, created by the Multilateral Working Group on Water Resources of the Middle East Peace Process. Its mission is to conduct, facilitate, coordinate, and support state-of-the-art basic and applied research, including training and educational programmes, and encourage the sharing of relevant information through electronic networking, with special emphasis on the needs of the Middle East region. The particular concern of the Centre will be desalination issues.

The Centre Director will have overall executive responsibility for the institutional development and day to day running of the Centre and will be accountable directly to the Board of Governors.

Since the Centre is an entirely new project, it is essential that its first director should have the vision and energy to establish its profile internationally, and to secure lasting financial support for its activity.

The Centre Director should certainly possess:
- proven management and leadership skills
- experience of institutional development in a developing nation context,
- verbal and written communication skills in English,
- certain expertise in the field of water treatment or process engineering.

Salary will be commensurate with experience. A generous benefits and relocation package will also be provided.

Suitably qualified candidates should forward their curriculum vitae and letter of interest stating their motivation for applying for this position to: **Science, Box 62, 1333 H Street NW, Room 814, Washington, DC 20005**

The deadline for receipt for all replies is December 31, 1995.

POSITIONS OPEN

HARVARD MEDICAL SCHOOL
HUMAN GENE THERAPY

Two **POSTDOCTORAL POSITIONS** to develop anti-tumor chimeric IgTCR-modified T cells for therapy. 1) Excellent molecular biology skills and/or retroviral vector experience. 2) Expertise in immunology and (optimally) mouse models; medical, veterinary or pathology background a plus. Send résumé, description of prior research, and names of at least two references by **FAX: 617-632-0998** to: **R. P. Junghans, Ph.D., M.D., Director, Biotherapeutics Development Lab.**

FELLOWSHIP IN
EXPERIMENTAL NEURO-ONCOLOGY

Two one to two year **FELLOWSHIPS** in Experimental Neuro-Oncology are offered for qualified M.D., Ph.D., and M.D./Ph.D. applicants interested in a research career in Neuro-Oncology. Individuals will work with and in the laboratory of a specific scientist within the Neuro-Oncology Program of the Mayo Comprehensive Cancer Center, Rochester, Minnesota. The areas of potential investigation include, but are not limited to, signal transduction, molecular genetics, developmental pharmacology, and experimental epidemiology. Interested individuals should send copies of their curriculum vitae and bibliography and two letters of reference to: **B. P. O'Neill, M.D., Department of Neurology, Mayo Clinic, 200 First Street SW, Rochester, MN 55905.** *Mayo Foundation is an Affirmative Action and Equal Opportunity Educator and Employer.*

NATIONAL ZOOLOGICAL PARK
SMITHSONIAN INSTITUTION
Postdoctoral and Predoctoral Fellowships

FELLOWSHIPS are available for research to be conducted at the National Zoological Park in animal behavior, animal medicine, conservation biology, evolution, ecology, genetics, nutrition, pathology, and reproductive physiology. One postdoctoral fellowship in 1996 is limited to studies using molecular methods (contact: **Rob Fleischer, National Zoological Park, Smithsonian Institution, Washington, DC 20008. Email: NZPGL100@SIVM.SI.EDU**). Stipends are $25,000 annually plus research expenses; tenures from three to 12 months (or up to 24 months for the molecular postdoc). Deadline is 15 January 1996. For information and application materials write: **Office of Fellowships and Grants, Desk MS, Smithsonian Institution, Washington, DC 20560.**

RESEARCH SCIENTIST
POSTDOCTORAL FELLOW

A position is available in our laboratory for a highly motivated scientist with extensive experience in the culture of mammalian cell lines. We are especially interested in individuals who have expertise in cell transfection, cell proliferation and transformation assays, and the establishment of primary cell lines from benign and malignant human tissue specimens. Additional expertise in either molecular biology or protein biochemistry would be extremely helpful. Our laboratory has identified a number of genes whose expression is altered during breast cancer initiation or progression. The qualified individual will be responsible for determining the oncogenic potential of these genes and will receive a highly competitive salary and benefits package not contingent on grants. Please send curriculum vitae and the names of two references to: **Scott Shepard, Ph.D., Pathology Department, Faulkner Hospital, 1153 Centre Street, Boston, MA 02130.**

COURSES AND TRAINING

HOW TO MAKE MICROCAPSULES
A HANDS-ON COURSE

A laboratory course designed to teach the art and science of microcapsule making. Suitable for biologists, pharmacists, chemists, life scientists, and engineers. Enrollment limited to 12. Lectures and laboratory sessions by **Dr. Curt Thies,** Professor of Chemical Engineering at Washington University in St. Louis. Fall Sessions: November 5, 6, 7, and 8, 1995. Spring Sessions: February 5, 6, 7, and 8; April 14, 15, 16, and 17, 1996. For more information **Telephone: 314-935-6082.**

SYMPOSIUM

URGENT NOTICE

We regret to announce that the Third Annual HERL Risk Assessment Symposium on Susceptibility and Risk **MUST BE POSTPONED OR CANCELLED** due to severe budgetary constraints during this period. The conference was originally scheduled for November 6 through 9, 1995 in Raleigh, North Carolina. We are investigating the availability of dates in the spring of 1996. The new date will be broadcast widely through mailings and advertisements. We apologize for the inconvenience this may cause, and hope to reschedule for a time that is convenient for speakers and attendees alike.

COURSES AND TRAINING

IMMUNOGENETICIST

The University of Puget Sound Biology Department seeks an **ASSISTANT PROFESSOR** for a tenure-track position (to begin Fall Term 1996) to teach lectures and labs for a one-semester junior-level course in genetics and an upper-level elective course in immunology. Duties will also include teaching introductory biology for majors or non-majors. Faculty are expected to establish a research program that involves undergraduates. Ph.D. required; postdoctoral experience preferred. Candidates should submit interest letter (including statements of teaching and research interests), curriculum vitae, and three reference letters that evaluate teaching and research, to be postmarked by November 17, 1995, to: **Biology Search, University of Puget Sound, P.O. Box 7297, Tacoma, WA 98407.** *An Equal Opportunity/Affirmative Action Educator/Employer.*

U.S. POSTAL SERVICE

Statement required by the Act of 12 August 1970, Section 3685, Title 39, United States Code, showing the ownership, management, and circulation of:

1-9. *Science*, Publication No. 0036-8075, is published weekly on Friday, except the last week in December, at 1333 H Street, NW, Washington, DC 20005–4792. Date of filing: 29 September 1995. This is also the address of the publisher, the editor, and the managing editor, who are, respectively, Richard S. Nicholson, Floyd Bloom, and Monica M. Bradford.

10. The owner is the American Association for the Advancement of Science, 1333 H Street, NW, Washington, DC 20005–4792. Stockholders: None.

11. Known bondholders, mortgages, and other security holders owning or holding 1 percent or more of total amount of bonds, mortgages, or other securities: None.

12. The purpose, function, and nonprofit status of this organization and the exempt status for federal income tax purposes have not changed during the preceding 12 months.

13–15. The average number of copies of each issue during the preceding 12 months is (A) Total number of copies printed: 171,485; (B) Paid circulation: (1) Sales through dealers and carriers, street vendors, counter sales: None; (2) Mail subscriptions: 162,677; (C) Total paid circulation: 162,677; (D) Free distribution by mail: Samples, complimentary, and other free copies: 3,779; (E) Free distribution outside of mail: Carrier or other means: 1,787; (F) Total free distribution: 5,566; (G) Total distribution: 168,243; (H) Copies not distributed: (1) Office use, leftover, unaccounted, spoiled after printing 3,242; (2) Returns from news agents: None; (I) Total: 171,485. Percent paid and/or Requested Circulation: 96.7%

Actual number of copies of single issue published nearest to filing date are (A) Total number of copies printed: 169,500; (B) Paid circulation: (1) Sales through dealers and carriers, street vendors, counter sales: None; (2) Mail subscriptions: 159,096; (C) Total paid circulation: 159,096; (D) Free distribution by mail: Samples, complimentary, and other free copies: 3,738; (E) Free distribution outside of mail: Carrier or other means: 4,200; (F) Total free distribution: 7,938; (G) Total distribution 167,034; (H) Copies not distributed: (1) Office use, leftover, unaccounted, spoiled after printing: 2,466; (2) Returns from news agents: None; (I) Total: 169,500. Percent paid and/or Requested Circulation: 95.3%

I certify that the statements made by me above are correct and complete. **(signed)** *Michael Spinella,* Director, Membership/Circulation.

COMPUTER MARKETPLACE

MARKETPLACE

Marketplace ads continue on next page

Marketplace continued from page 545

NOW THAT OPERON'S DNA *is just* $1.20 PER BASE, YOUR *savings* ARE SURE TO multiply.

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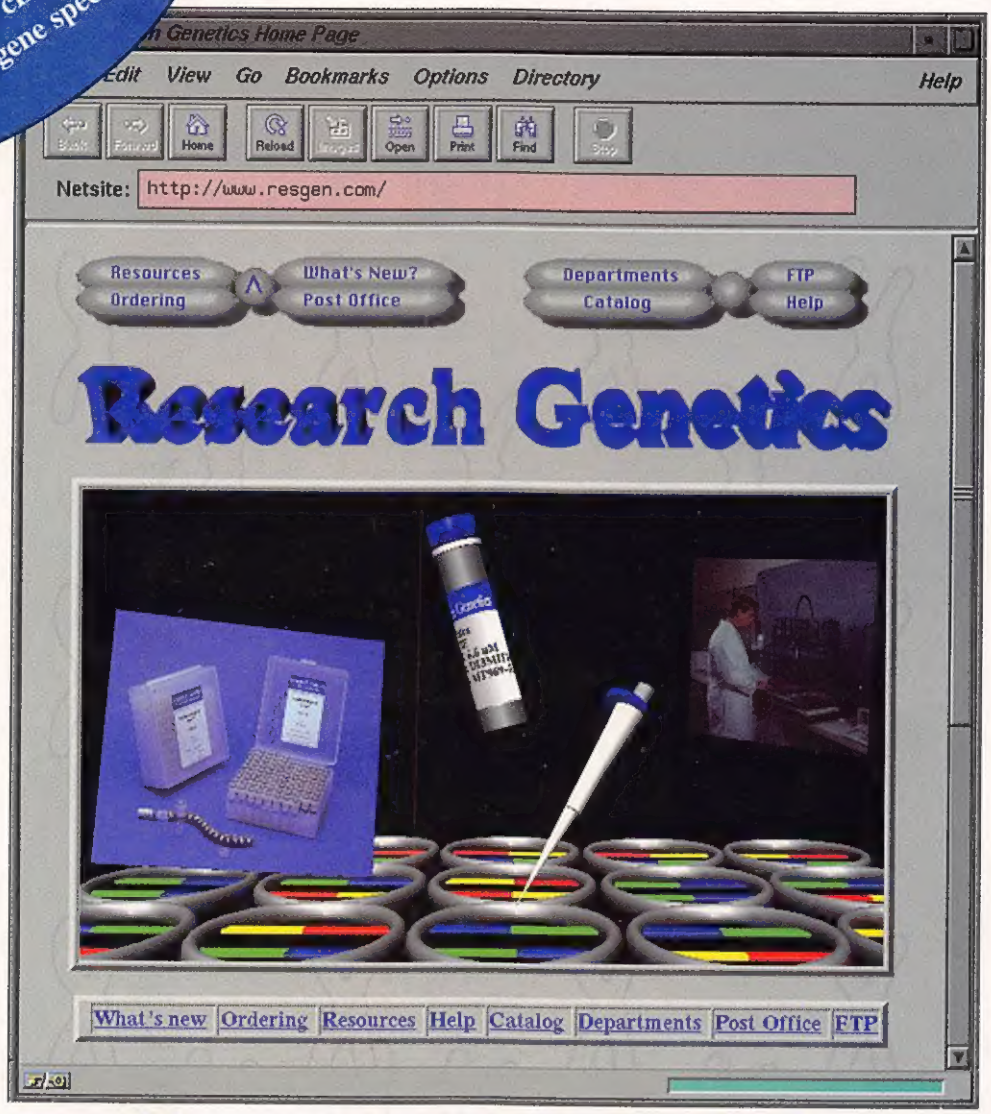

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